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ORIGINAL ARTICLE
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Beat-to-Beat Variability in Preload Unmasks Latent Risk of Torsade de Pointes in Anesthetized Chronic Atrioventricular Block Dogs

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Background: Beat-to-beat variability in ventricular repolarization (BVR) associates with increased arrhythmic risk. Proarrhythmic remodeling in the dog with chronic AV-block (CAVB) compromises repolarization reserve and associates with increased BVR, which further increases upon dofetilide infusion and correlates with Torsade de Pointes (TdP) arrhythmias. It was hypothesized that these pro-arrhythmia-associated increases in BVR are induced by beat-to-beat variability in preload.

Methods and Results: Left ventricular monophasic action potential duration (LVMAPD) was recorded in acute (AAVB) and CAVB dogs, before and after dofetilide infusion. BVR was quantified as short-term variability of LVMAPD. The PQ-interval was controlled by pacing: either a constant or an alternating preload pattern was established, verified by PV-loop. The effect of the stretch-activated channel blocker, streptomycin, on BVR was evaluated in a second CAVB group. At alternating preload only, BVR was increased after proarrhythmic remodeling (0.45±0.14 ms AAVB vs. 2.2±1.1 ms CAVB, P<0.01). At CAVB, but not at AAVB, dofetilide induced significant proarrhythmia. Preload variability augmented the dofetilide-induced BVR increase at CAVB (+1.5±0.8 ms vs. +0.9±0.9 ms, P=0.058). In the second group, the increase in baseline BVR by alternating preload (0.3±0.03 ms to 1.0±0.8 ms, P<0.01) was abolished by streptomycin (0.5±0.2 ms, P<0.05).

Conclusions: In CAVB dogs, the inverse relation between BVR and repolarization reserve originates from an augmented sensitivity of ventricular repolarization to beat-to-beat preload changes. Stretch-activated channels appear to be involved in the mechanism of BVR. (*Circ J* 2016; **80:** 1336–1345)

Key Words: Electrical remodeling; Preload; Short-term variability of repolarization; Stretch; Torsade de Pointes

Sudden cardiac death is a major cause of mortality in the general population.[1] Temporal variability of repolarization has been quantified in different ways and shown to be higher in patients with increased risk of arrhythmias.[2–11] The occurrence of repolarization variability is often interpreted as lability of repolarization, resulting from a reduction in the excess capacity of repolarizing currents, known as the 'reduced repolarization reserve' concept.[12] The positive correlation between repolarization variability and arrhythmogenicity is also found in experimental models of cardiac arrhythmia; in animal models sensitive to drug-induced Torsade de Pointes (TdP), beat-to-beat variability of repolarization duration (BVR) is used as a predictor of proarrhythmic side-effects in pre-clinical drug screening.[13–15] Thus far, the mechanisms underlying BVR are not completely understood, which brought us to the current study.

In the canine chronic AV-block (CAVB) model, an increased TdP-susceptibility is closely linked to a process of ventricular remodeling, which results from the chronic bradycardia and altered ventricular activation with AV-dyssynchrony.[16,17] This remodeling results in prolonged repolarization time, but also increased BVR, quantified as short-term variability (STV) of left ventricular (LV) monophasic action potential duration (MAPD) [STV_{MAPD}].[16] Drugs that further prolonged repolar-

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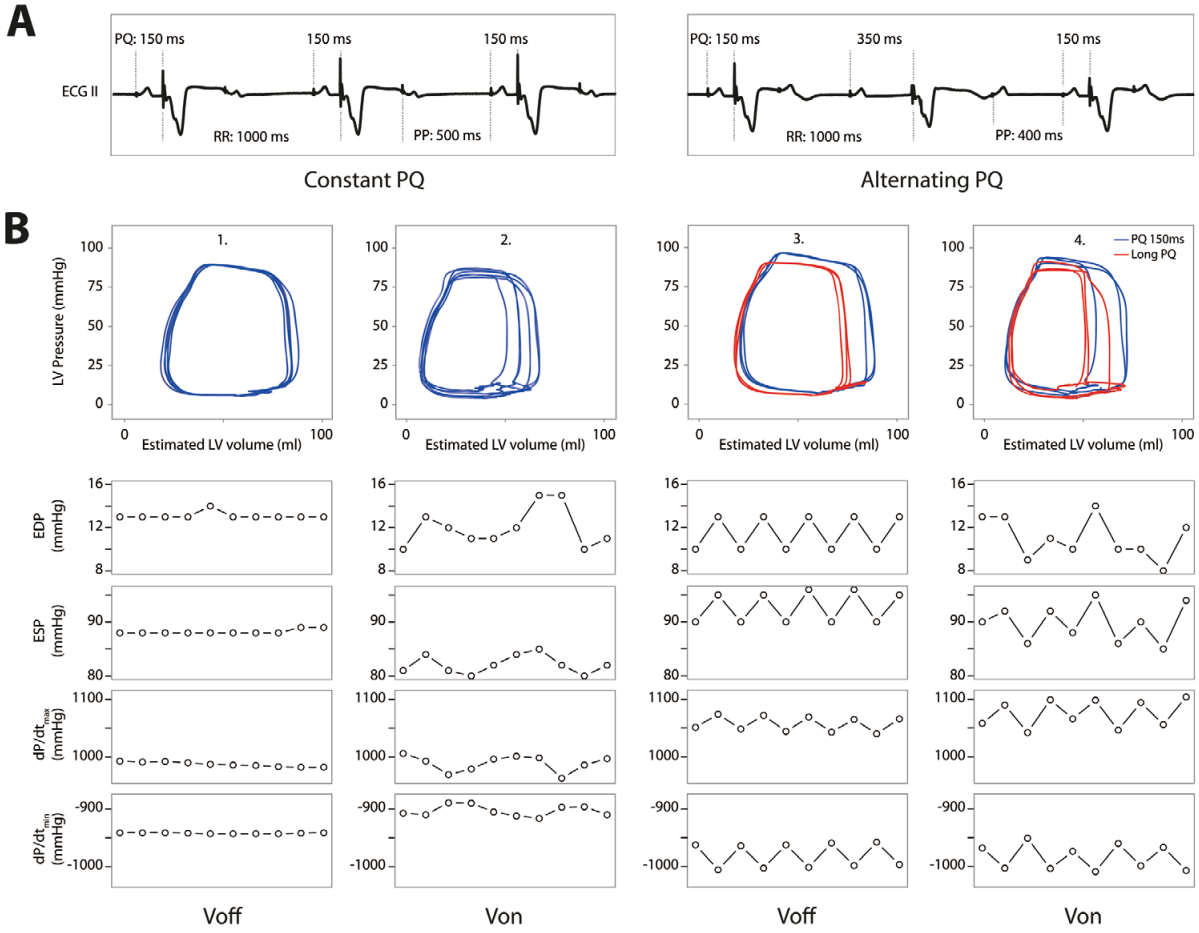

Figure 1. (**A**) Example ECG representation of pacing protocols used. Stimulation of atria and ventricles at different heart rates allowed control of atrioventricular timing, while preserving the ventricular bradycardia required for Torsade de Pointes (TdP) induction. When the paced VV (RR) interval was an exact multiple of the AA (PP) interval, each ventricular activation is preceded by the same PQ interval (left tracing), whereas an RR:PP-ratio of 2.5 resulted in PQ interval alternation (right tracing). Printed numbers are resulting PP, PQ and RR intervals. (**B**) Pressure-volume (PV) loop recordings, each showing 6 consecutive beats during different protocols in which preload variability was acutely controlled. The lower four panels show parameters derived from the pressure recording over 10 consecutive beats. Clearly visible is the minimal beat-to-beat mechanical variation during constant PQ with ventilation switched off (left PV loop). Alternating PQ results in a reproducible alternating PV-loop pattern, but also of the other mechanical parameters and volume (PV loop 3) is shown. Ventilation alone (PV loop 2) results in cyclical variation of end-diastolic pressure (EDP). The cycle length corresponds to the ventilation frequency of 12/min. During alternating PQ with ventilation switched on, the alternating pattern still dominates (PV loop 4).

ization and induced TdP yielded a strong increase in BVR just prior to the arrhythmia occurrence, whereas non-arrhythmogenic drugs, despite QT prolongation, did not increase BVR.[18–21] Besides myocardial remodeling effects, additional factors like preload may also contribute to BVR. Preload causes stretch, and stretch in general is known to influence cardiac electrophysiology and its underlying factors.[22–24] This may be especially true in the AV-block dog, in which AV-dyssynchrony leads to uncontrolled beat-to-beat changes in ventricular preload. We hypothesized that in a situation of reduced repolarization reserve, this variability in preload is required to provoke repolarization variability, and thus unmasks a latent risk for drug-induced TdP arrhythmias.

In the current study, we investigated the influence of preload variability resulting from AV-dyssynchrony, on BVR in 3 experimental situations with different degrees of repolarization reserve (healthy and remodeled heart, and remodeling+I_{Kr} blockade). Furthermore, we evaluated the effect of stretch-activated channel (SAC) block on both BVR and the induction of TdP by I_{Kr}-block. Results show that preload variability is required for BVR to increase after proarrhythmic remodeling or I_{Kr}-block, and that reduction of stretch activated current (I_{SAC}) will attenuate both baseline BVR and arrhythmic response to dofetilide.

Methods

Animal Handling

All experiments were approved by the committee for experiments on animals of Utrecht University (application 2008. II.05.046), and animal handling was in accordance with the Dutch Law on Animal Experimentation and the European

Table 1. Parameters Derived From Pressure-Volume Loop Recordings During the Different Protocols That Were Used to Control Preload Variability

	Constant PQ		Alternating PQ	
	Ventilation off	Ventilation on	Ventilation off	Ventilation on
STV_{EDP}, mmHg	0.1±0.0	0.9±0.2***	1.2±0.7***	1.3±0.3
STV_{ESP}, mmHg	0.3±0.3	1.1±0.4***	3.0±1.1***	3.0±1.0†††
$STV_{dP/dt\ max}$, mmHg/s	1.6±0.6	5.0±3.7***	16.8±8.3***	17.0±9.3††
$STV_{dP/dt\ min}$, mmHg/s	1.2±0.5	4.2±1.6***	13.1±7.5***	15.1±8.4†
STV_{EDV}, mmHg	*0.8±0.3*	*2.2±1.4****	*3.7±2.2****	*3.6±1.7††*
STV_{ESV}, mmHg	*0.7±0.3*	*1.2±0.5****	*2.7±1.2***	*2.5±1.2†*
STV_{SW}, mmHg	*83±23*	*157±124****	*370±221****	*336±215††*
EDP, mmHg	14.1±4.1	14.4±4.1	13.3±4.0*	13.2±3.9†
PQ 150 ms	–	–	14.1±4.0	13.9±4.1
PQ ≥350 ms	–	–	12.4±4.0‡‡	12.6±3.8‡‡‡
ESP, mmHg	92±6	91±6	90±5	89±4
dP/dt_{max}, mmHg/s	966±158	960±161	965±150	969±158
dP/dt_{min}, mmHg/s	−893±147	−885±144	−896±127	−891±119
EDV, ml	*80±23*	*75±30*	*82±28*	*77±31*
PQ 150 ms	–	–	*84±27*	*79±30*
PQ ≥350 ms	–	–	*79±29‡‡*	*74±31‡‡*
ESV, ml	*34±22*	*29±26*	*34±25*	*30±28*
SW, ml·mmHg	*4,047±712*	*3,958±787*	*4,076±688*	*3,991±761*

The four different protocols are explained by an individual example in Figure 1. Parameters in italics are estimates only, because a pseudo-calibration was used for the volume signal. During the measurements, the ventricle was paced at a fixed rate of 60/min, while the PQ was either constant (150 ms) or alternating (between 150 and >350 ms; see also Figure 1). Measurements were performed with and without mechanical ventilation. STV, short-term variability; EDP, end-diastolic pressure; ESP, end-systolic pressure; dP/dt_{max}, maximum rate of rise of LV pressure; dP/dt_{min}, minimum rate of fall of left ventricular pressure; EDV, end-diastolic volume; ESV, end-systolic volume; SW, stroke work. *,**,***P<0.05/<0.01/<0.001 vs. constant PQ and ventilation off. †,††,†††P<0.05/<0.01/<0.001 vs. constant PQ and ventilation on. No differences were found between ventilation on and ventilation off during alternating PQ (2-way repeated measures ANOVA with post-hoc comparisons with Bonferroni correction). ‡‡,‡‡‡P<0.01/<0.001 vs. PQ 150 ms (paired Student's t-test (EDP) or Wilcoxon signed-ranks test (EDV)). Values are presented as mean ± SD; n=8.

Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Union Directive 86/609/EEC).

Further details on animal handling, preparation, data acquisition, data analysis and statistics are described in the supplementary material.

Pacing-Induced Preload Variability

Dyssynchrony of atrial and ventricular contractions is typical for the AV-block model and results in beat-to-beat changes in preload. We designed a pacing protocol to control atrial and ventricular activation separately, allowing us to switch preload variability on or off. An external pacemaker (PK5; Vitatron, Arnhem, The Netherlands) was adapted to perform stimulation of atria and ventricles at different pacing rates, starting at a pre-set AV delay (=PQ interval on ECG) of 150 ms (similar to sinus rhythm). This allowed control of the PQ interval, and thereby preload, while maintaining the ventricular bradycardia required for TdP induction at 1,000 ms.[25] The pacing sequences are illustrated in **Figure 1A**: a constant PQ interval (constant preload) was obtained when the RR:PP-ratio was 2.0 (or 3.0), while an RR:PP-ratio of 2.5 (or 3.5) provoked an alternating PQ interval (maximal beat-to-beat variation), resulting in maximum pacing-induced preload variability (**Figure 1B**).

Because ventilation caused alterations in preload as well, measurements were preferably performed with ventilation temporarily switched off. Only the effect of dofetilide, where STV_{MAPD} is evaluated just before first ectopic activity, was evaluated with ventilation on. The effects of alternating PQ, constant PQ and mechanical ventilation (on and off, Von and Voff, respectively) on preload and BVR were evaluated (4 modes of preload variation, **Figure 1B**). Volume-controlled mechanical ventilation was set at a frequency of one-fifth of the heart rate. During Voff, mechanical ventilation was ceased for 30s.

Challenges on Repolarization Reserve: Cardiac Remodeling and I_{Kr} Block

To compare the effect of preload variability on BVR after various challenges on repolarization reserve, 11 animals were used (age 1.3±0.3 years, body weight 22±3 kg, 6 males, mongrel; Marshall, NY, USA) and baseline BVR was measured at acute AV-block (AAVB) in 6 dogs. Five animals also received a dofetilide challenge (0.025 mg/kg in 5 min) to test susceptibility to TdP after I_{Kr} block. Measurements with dofetilide at AAVB were randomized to with or without pacing-induced preload variability.

Serial experiments in the remodeled heart,[26] were performed in 7 animals at 3 and 5 weeks CAVB. Both the effects of preload variability on BVR at baseline and after administration of dofetilide were tested; once with pacing-induced preload variability (alternating PQ) and once without (constant PQ), in a random order.

After the start of dofetilide infusion, the electrocardiogram (ECG) was monitored for TdP, which was defined as a polymorphic ventricular tachyarrhythmia of at least 5 beats characterized by a twisting shape of the QRS complexes around the isoelectric line. Dofetilide infusion was stopped after the first detected TdP episode. If ventricular tachycardia lasted more than 10 s, electrical cardioversion was applied (10–150–200J biphasic).

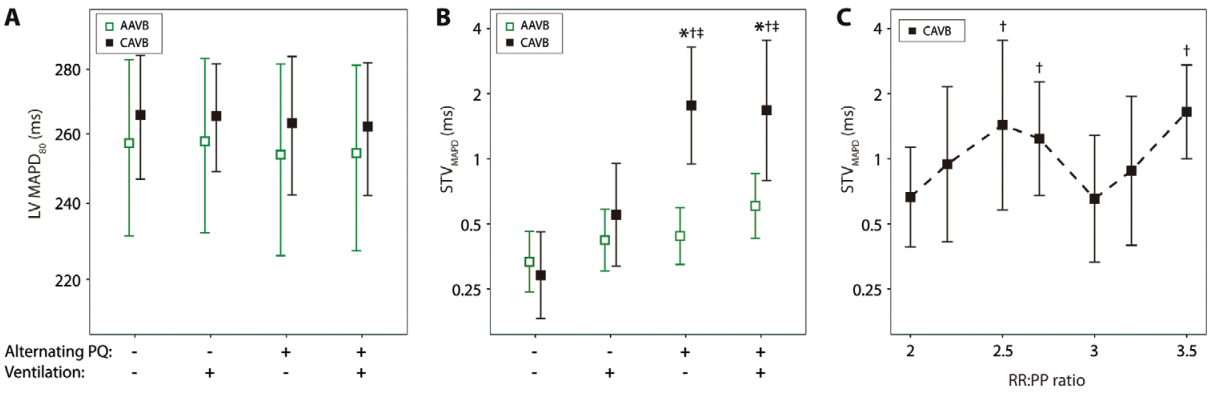

Figure 2. Effect of beat-to-beat changes in preload caused by PQ variation and mechanical ventilation on (**A**) left ventricular monophasic action potential duration (LV MAPD) and (**B**) short-term variability of LV MAPD (STV_{MAPD}) at acute and chronic AV-block (AAVB, CAVB). *P<0.05 vs. AAVB; †P<0.05 vs. constant PQ and ventilation switched off; ‡P<0.05 vs. constant PQ and ventilation switched on (2-way repeated measures ANOVA with post-hoc comparisons with Bonferroni correction). (**C**) Effect of variations in the ratio of ventricular to atrial cycle length (RR:PP ratio). The atrial cycle length was stepwise shortened, while maintaining the fixed RR interval of 1,000 ms. Maxima for STV_{MAPD} are seen at alternating PQ (ratio 2.5 and 3.5), whereas minima are observed at 2.0 and 3.0, where PQ is constant. †P<0.05 vs. 2.0 (1-way repeated measures with post-hoc comparisons with Bonferroni correction vs. 2.0). Values are presented as mean±standard deviation (SD) (**A**; n=6) or geometric mean and SD (**B** and **C**; each n=6 dogs). Note the logarithmic Y-axis in (**B**) and (**C**).

Quantification of Preload Variability and Effect of SAC Block

Pressure-volume (PV) loops were recorded in order to quantify the pacing-induced preload variability, with and without mechanical ventilation, simultaneously with LV MAP, in an additional set of 9 CAVB dogs (median 9 [7–14] weeks after AV-block). In the same experiment, the effect of SAC block by streptomycin on baseline BVR and arrhythmic response to dofetilide was tested; during pacing with alternating PQ, streptomycin was infused (40 mg/kg in 5 min i.v.), followed 10 min later by dofetilide (0.025 mg/kg in 5 min).

Arrhythmic Score

Arrhythmic outcome was quantified by combining the number of ectopic beats, episodes of TdP and defibrillations into a single arrhythmia score. Each regular beat during the 10-min interval after dofetilide administration was scored 1 point by default. Single ectopic beats, initiated within the T wave, were scored 2 points, while runs of polymorphic ectopic beats were score 3–50 points, corresponding to the number of beats. Scores of 50, 75 and 100 were applied if a single arrhythmic episode required 1, 2 or ≥3 defibrillations. Arrhythmia score was calculated as the mean of the 3 highest scores.

Results

Controlled Variation in PQ Results in Reproducible Preload Variability

To determine beat-to-beat effects of pacing-induced preload variability, STV calculations of LV mechanical parameters derived from PV loop recordings (individual example in **Figure 1B**) were determined (upper part **Table 1**). While ventilation was off, alternating PQ resulted in a significant increase in STV of all pressure-derived parameters compared to constant PQ pacing, showing that we have ample control of preload variability through our stimulation protocols. No relevant difference was observed when ventilation was switched on. Looking at individual beats during alternating PQ (ventilation off), end-diastolic pressure (EDP) and end-diastolic volume were consistently and significantly decreased during the beats with long PQ (>350 ms) compared to beats with short PQ (150 ms) (lower part **Table 1**). Similar effects were seen for all other hemodynamic parameters (data not shown). Apart from a small decrease in EDP at alternating PQ, the 30-beats averages of the mechanical parameters were not influenced by the 4 modes of preload variability (lower part **Table 1**), illustrating no pacing-induced effects on cardiac output.

Detection of Proarrhythmic Remodeling Through Increased BVR Requires Preload Variability

As expected, ventricular remodeling due to CAVB at idioventricular rhythm was associated with QT_c prolongation: 327±19 ms in AAVB and 440±46 ms in CAVB dogs (P=0.001). At AAVB, LV MAPD and STV_{MAPD} were not affected by changes in preload variability (**Figures 2A**,**2B**, open squares). However, in CAVB hearts, the electrical remodeling was associated with significantly increased baseline STV_{MAPD}, but only during pacing with alternating PQ interval (**Figure 2B**, black squares). We consistently observed a longer LV MAPD in beats with short PQ (highest preload). Mechanical ventilation had no significant effect on STV_{MAPD} either before or after remodeling (**Figure 2B**).

To confirm that the effect on STV_{MAPD} at CAVB was caused by PQ variability and not by the PP interval itself, we recorded STV_{MAPD} at a range of PP intervals, resulting in PP:RR ratios from 2.0 to 3.5 (**Figure 2C**). The local minima at constant PQ (ratios 2.0 and 3.0) and local maxima at alternating PQ (ratios 2.5 and 3.5), but no trend in STV_{MAPD} from left to right (decreasing PP), show that STV_{MAPD} is indeed the result of the variation of PQ interval and not of PP interval per se.

Preload Variability Augments STV Increase After Dofetilide

Consistent with previous publications, administration of dofetilide induced an arrhythmic response in CAVB (**Figure 3A**),

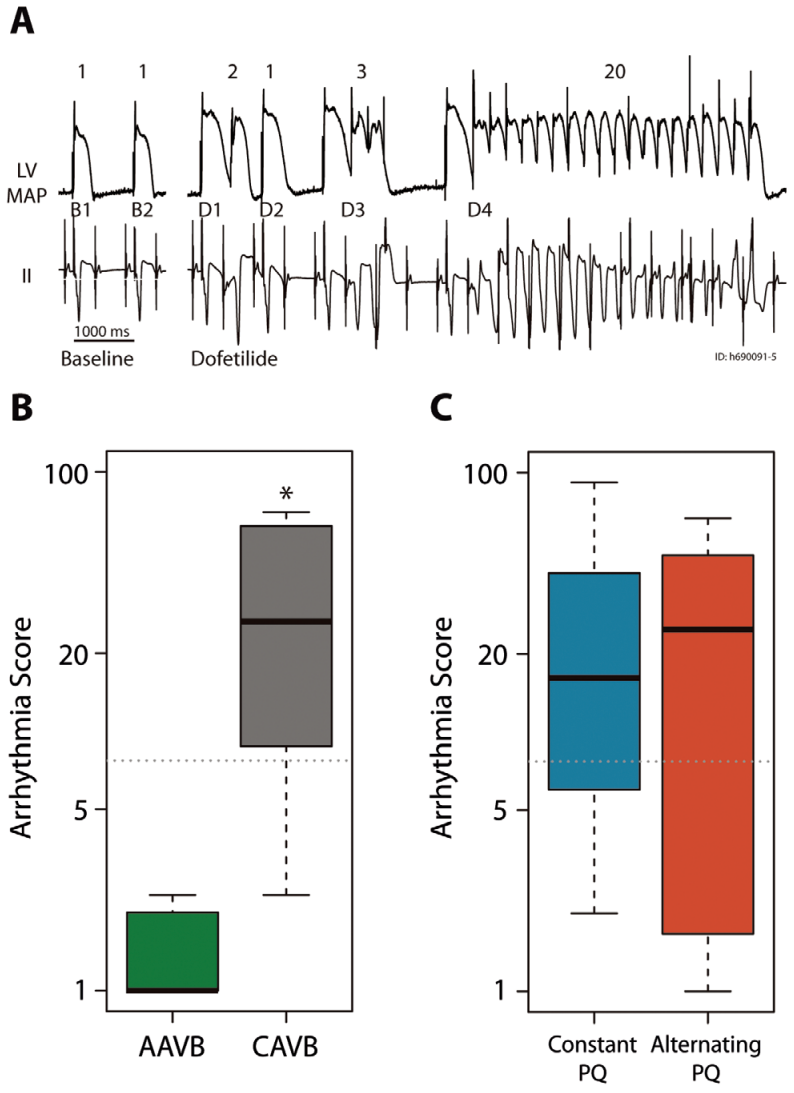

Figure 3. Effect of dofetilide on arrhythmia score. (**A**) Individual example of electrophysiological recordings showing, on the left, 2 beats at baseline immediately prior to the start of dofetilide infusion, and on the right, an example of dofetilide-induced arrhythmias. In this experiment, the PQ interval was kept constant at 150 ms. The heart was paced at a ventricular rate of 60 beats/min and an atrial rate of 120 beats/min (PP:RR=1:2). The upper tracing shows the left ventricular monophasic action potential (LV MAP), the lower tracing shows the ECG lead II. During dofetilide, four ventricular-paced beats are depicted (D1-4), of which D1, D3 and D4 are followed by ectopic beats. These arrhythmias are classified as single ectopic beat (D1), multiple ectopic beat (D3) and episode of Torsade de Pointes (TdP; D4). The corresponding scores (used for the calculation of the arrhythmia score) are shown at the top of the figure. (**B**) In unremodeled dogs at acute AV-block (AAVB; n=5), dofetilide was administered during either alternating (n=3) or constant PQ (n=2). TdP was not observed, resulting in a low arrhythmia score. At chronic AV-block (CAVB; n=7), dogs were tested twice, at both alternating PQ and at constant PQ (1 missing). The mean arrhythmia score of these 2 experiments was higher than at AAVB (P=0.005, Wilcoxon rank-sum test). (**C**) Serial analysis of arrhythmia score after dofetilide during alternating and constant PQ revealed no difference (P=1.00, Wilcoxon signed-rank test). Note: the dotted line marks the cut-off value to discriminate TdP inducible vs. non-inducible CAVB animals (based on at least 3 TdP episodes).

but not in AAVB; pooled results for experiments with constant and alternating PQ (Figure 3B) show that the arrhythmia score was significantly higher in CAVB dogs (AAVB: 1 [1–2], (n=5); CAVB: 27 [9–62] (n=7); P=0.005). When comparing the serial experiments (constant and alternating PQ) at CAVB, it is clear that the arrhythmia score was not changed (16 [7–37] at constant PQ; 25 [2–48] at alternating PQ, n=6; P=1.00; Figure 3C). Thus, the presence of a latent risk for TdP arrhythmias in CAVB compared to AAVB can be assessed by preload variability-mediated increases in BVR (Figure 2B), while the pacing-induced preload variability itself did not contribute to drug-induced proarrhythmia.

Dofetilide increased LV MAPD equally during constant PQ and alternating PQ, and no significant differences were present between the groups (Figure 4A). Mechanical ventilation was used during the dofetilide challenge, resulting in minor beat-to-beat preload variation in the experiment with constant PQ as well. However, STV_{MAPD} still showed a trend towards a higher increase at alternating PQ (Figure 4B). After detrending MAPD to correct for the progressive prolongation by dofetilide, the STV increase at alternating PQ was significantly augmented (+5.7±4.4 ms alternating PQ vs. +0.6±0.6 ms constant PQ, P=0.015, paired t-test after log-transformation). In Figure 4C, an individual example is shown; clearly visible are the oscillations in MAPD corresponding to alternating PQ that are augmented after dofetilide, while at constant PQ, beat-to-beat variability after dofetilide is dominated by the progressive prolongation of LV MAPD.

Streptomycin Abolishes Response of BVR to Preload Variation and Attenuates Arrhythmic Response to Dofetilide

The response of STV_{MAPD} to alternating PQ at baseline was abolished by streptomycin (individual example in Figure 5): from 0.3±0.03 ms at constant PQ to 1.0±0.8 ms at alternating PQ (P<0.01) back to 0.5±0.2 ms at alternating PQ after streptomycin (P<0.05 vs. baseline alternating, NS vs. baseline constant). Streptomycin had no effect on the variability of the LV mechanical parameters, while the 30-beat averages only decreased slightly after the administration (Table 2).

Pretreatment with streptomycin showed a strong trend towards reduction of the arrhythmia score following dofetilide

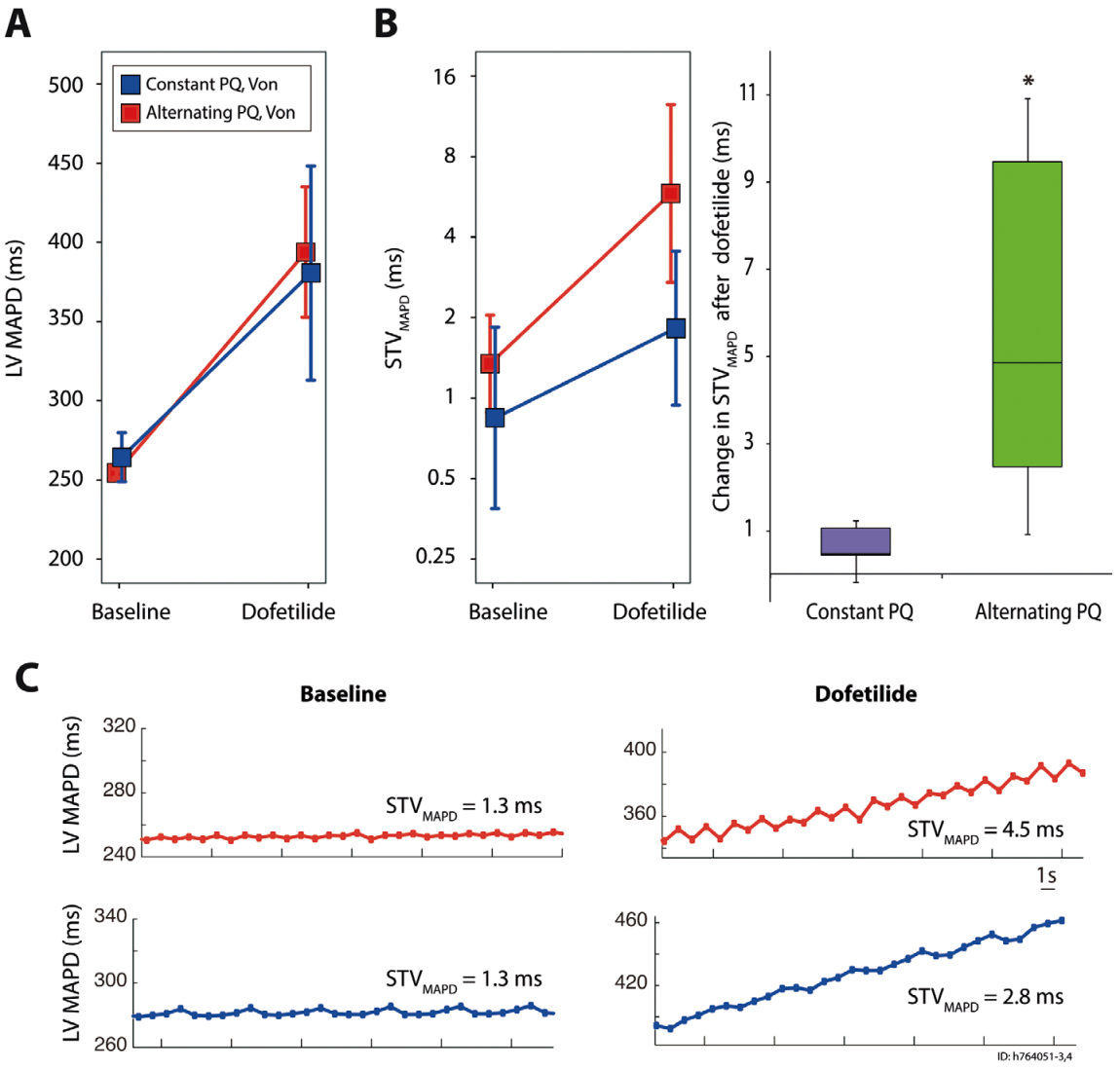

Figure 4. Electrophysiological effects of dofetilide during alternating PQ (red) and constant PQ (blue). (**A**) Immediately after complete infusion of dofetilide (or before induction of the first ectopic beat), left ventricular monophasic action potential duration (LV MAPD) is increased equally during constant and alternating PQ. (**B**) Left panel: The effect of dofetilide on short-term variability MAPD (STVMAPD) is shown as geometric mean and geometric SD, on a logarithmic scale. There was a trend towards an augmented increase of STVMAPD during alternating PQ vs. constant PQ (P=0.13, paired t-test after log-transformation; n=5). Right panel: The change in STV after dofetilide administration was significantly higher at alternating PQ when correcting for the trend in MAPD (+5.7±4.4 alternating vs. +0.6±0.6 ms constant PQ, P=0.015, paired t-test after log-transformation). (**C**) Example showing traces of LV MAPD used for STVMAPD calculation at baseline and after dofetilide administration just before induction of ectopic beats (each 31 consecutive beats). Dofetilide administration increased the amplitude of the alternating MAPD after dofetilide (upper graphs), and at constant PQ, the beat-to-beat variability is dominated by the progressive repolarization prolongation by dofetilide (lower graphs).

infusion: 27 [9–62] vs. 4 [2–13] (P=0.07, Figure 6). However, this could not prevent an increase of STVMAPD, from 0.6±0.2 ms to 4.0±3.3 ms (P=0.006), and is similar to the value obtained in the group that received only dofetilide.

Discussion

Main Findings

The most important findings of this study are: (1) in the chronic AV-block dog model, the typical preload variability due to variability of the PQ interval is an important contributor to BVR; (2) this variation in preload is a prerequisite to obtain a significant increase of STVMAPD after proarrhythmic remodeling, and it augments the STVMAPD increase after dofetilide; and (3) the effect of preload variability on baseline STVMAPD is abolished by streptomycin infusion, while mechanical variation is maintained. These results confirm the association between STVMAPD and proarrhythmia, and suggest involvement of the SAC in BVR in the CAVB dog.

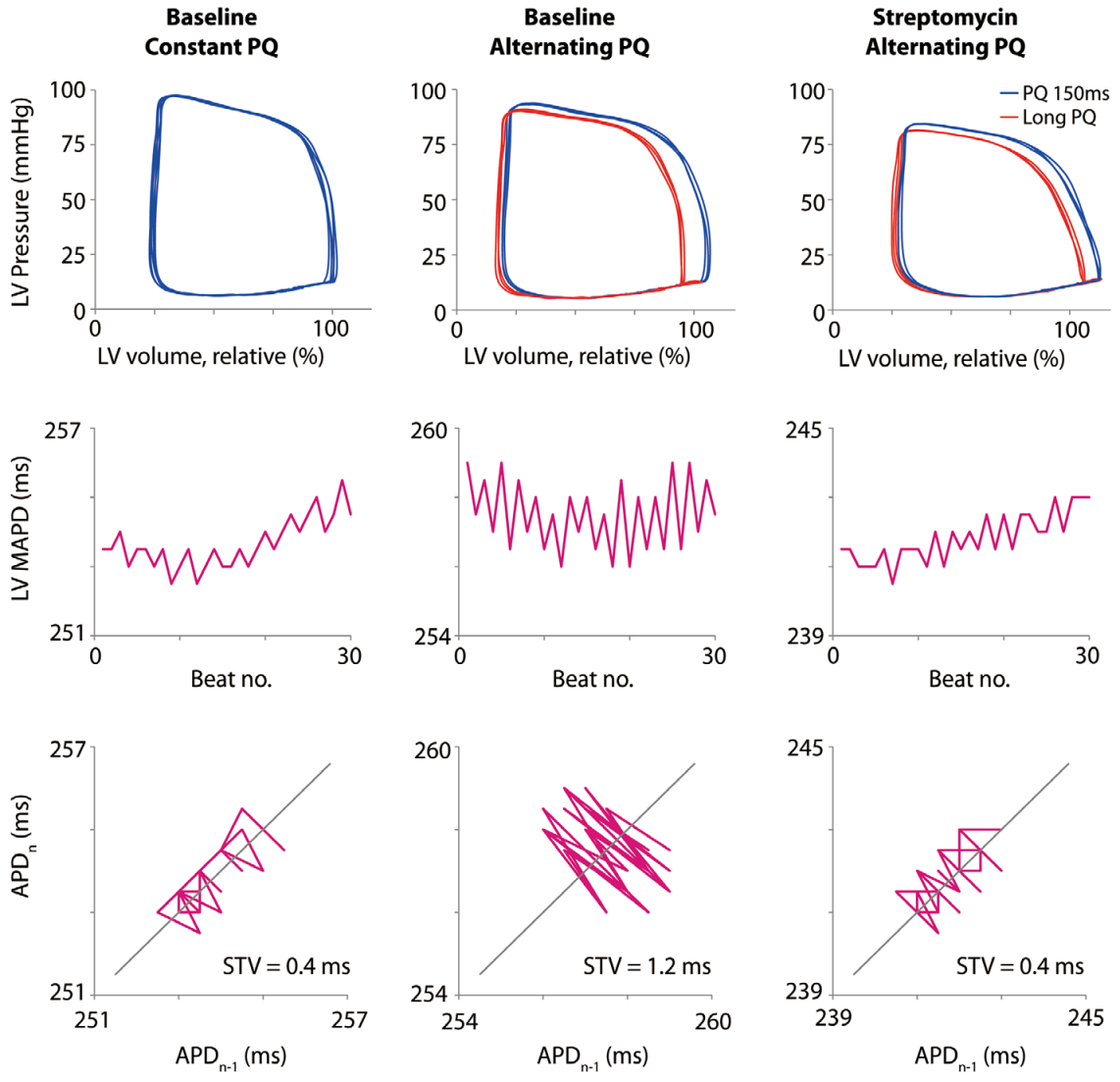

Figure 5. Example illustrating the streptomycin effect on the pressure-volume loop and on short-term variability of monophasic action potential duration (STV$_{MAPD}$, Poincaré plot in bottom row) in a dog with chronic AV-block. After streptomycin (**Right**), beat-to-beat alterations in preload are maintained, whereas STV$_{MAPD}$ is reduced to the same level as that seen during alternating PQ before streptomycin administration (**Left**). All measurements were performed with mechanical ventilation off.

Mechanistic Link Between BVR and Preload Variability

Previous publications have revealed the relation between ventricular remodeling, STV$_{MAPD}$ in baseline and the susceptibility to drug-induced arrhythmias in the anesthetized CAVB dog. In these CAVB dogs, baseline STV$_{MAPD}$ was increased in comparison to unremodeled hearts from dogs in sinus rhythm or in AAVB, and the highest baseline values were measured in animals in which TdP arrhythmias were inducible with dofetilide.[16] Induction of TdP was preceded by a further increase in STV. However, in these studies using the non-paced AV-block dog, the preload variability has not been controlled.

In the current study, we confirmed that baseline STV$_{MAPD}$ is increased after remodeling, but only if preload variability is present, and this effect was abolished after stretch-activated current (I_{SAC}) block. This dependence of STV on preload variability seems to continue after dofetilide. Thus, to unmask the lability of repolarization with baseline STV$_{MAPD}$, a (mechanical) 'challenge', here in the form of preload variability is essential. The simplest explanation would be that similar I_{SAC} would have a larger impact on repolarization under conditions of impaired repolarization reserve. In addition, I_{SAC} was found to be increased in cardiac hypertrophy,[27] which is in line with our findings on the role of I_{SAC} in STV.

The relative timing of electrical and mechanical systole may impact on BVR in the CAVB dog. Dofetilide prolongs repolarization, but does not delay the end of relaxation (causing a negative 'electromechanical window').[28,29] Isovolumic relaxation and filling during early diastole, which are associated with increased local differences in stretch, now overlap with the vulnerable phase of repolarization, allowing resulting I_{SAC} to affect the repolarization process locally. This mechano-electrical feedback may be enhanced by an additional factor

Table 2. Mechanical and Electrophysiological Effects of Preload Variability and the Effect of Streptomycin

	Baseline constant PQ	Baseline alternating PQ	Streptomycin alternating PQ
LV MAPD, ms	256±19	255±25	245±22
STV_{MAPD}, ms	0.3±0.1	1.2±0.7*	0.5±0.2†
STV_{EDP}, mmHg	0.1±0.0	1.2±0.6*	0.9±0.5*
STV_{ESP}, mmHg	0.3±0.3	2.8±0.9*	2.8±0.9*
$STV_{dP/dt\ max}$, mmHg/s	1.6±0.6	16.5±8.4*	22.0±17.5
$STV_{dP/dt\ min}$, mmHg/s	1.2±0.5	13.6±7.1*	12.1±11.2
STV_{EDV}, mmHg	*0.8±0.3*	*3.8±2.2**	*3.3±1.6**
STV_{ESV}, mmHg	*0.7±0.3*	*2.8±1.2**	*1.9±1.1**
STV_{SW}, ml·mmHg	*83±23*	*350±238**	*313±163**
EDP, mmHg	14.1±4.1	12.5±3.6	12.4±3.7
ESP, mmHg	92±6	87±7	79±9*
dP/dt_{max}, mmHg/s	966±158	913±166	780±137*,†
dP/dt_{min}, mmHg/s	−893±147	−864±135	−743±123*,†
EDV, ml	*80±23*	*67±3*	*71±4*
ESV, ml	*34±22*	*22±6*	*27±8*
SW, ml·mmHg	*4,047±712*	*3,815±734*	*2,990±517*

Parameters in italics are estimates only, because a pseudo-calibration was used for the volume signal. Mechanical parameters (pressure and volume) were derived from left ventricular pressure-volume loop recordings. LV MAPD, left ventricular monophasic action potential duration at 80% repolarization; STV_X, short term variability of parameter X. Other abbreviations as in Table 1. *P<0.05 vs. baseline during constant PQ; †P<0.05 vs. baseline during alternating PQ; (1-way repeated measures ANOVA or Friedman repeated measures ANOVA on ranks, and post hoc comparisons with Bonferroni correction). Values are presented as mean±SD; n=9 (pressure/volume: 1 missing).

such as increased I_{SAC} after remodeling.

Interpretations of Findings on the SAC Blockade by Streptomycin

To elucidate the role of the SAC in BVR, we tested the effect of I_{SAC} block by streptomycin (target plasma level 200 μmol/L) in CAVB dogs. While maintaining the mechanical beat-to-beat variation during alternating PQ, STV_{MAPD} returned to a level that had been observed at baseline during constant preload.

Streptomycin is a non-specific blocker of SAC (IC_{50}: 200 μmol/L). At higher concentrations, the drug will also block other channels, most importantly the L-type calcium channel (IC_{50} of I_{CaL}: 2 mmol/L).[30–32] In the CAVB model, I_{CaL} block is antiarrhythmic, but at a dose that compromises contractility, and it does not lower baseline STV_{MAPD}.[18,33] This argues for effective I_{SAC}-block by streptomycin as the cause of the reduction of baseline STV_{MAPD} in the current study. However, the negative inotropic effect of streptomycin (ESP reduction by 9%) may be an indication of I_{CaL}-block. Its extent is similar to that reported after an antiarrhythmic dose of verapamil, which blocks I_{CaL} but not I_{SAC}.[33]

The apparent antiarrhythmic effect of streptomycin against dofetilide-induced TdP suggests that SAC is involved in the arrhythmogenesis of TdP as well. Although here we cannot rule out that this is, in part, the effect of I_{CaL}-block by streptomycin. The antiarrhythmic effect was also observed in earlier experiments in CAVB dogs with uncontrolled preload (unpublished data). In these dogs, dofetilide infusion (0.025 mg·kg^{-1}·5 min^{-1}) was followed by streptomycin (40 mg·kg^{-1}·5 min^{-1}, n=7), while in controls (n=10), only dofetilide was administered. Arrhythmia score, determined during the 15–25 min interval after the start of dofetilide administration, was significantly lower in the streptomycin-treated animals (2 [1–3] vs. 4 [2–11], P=0.049). Literature provides a possible role for I_{SAC} in triggering of the ectopic beats that initiate the TdP arrhythmias; formation of early afterdepolarizations (EADs) in vivo may be a stochastic

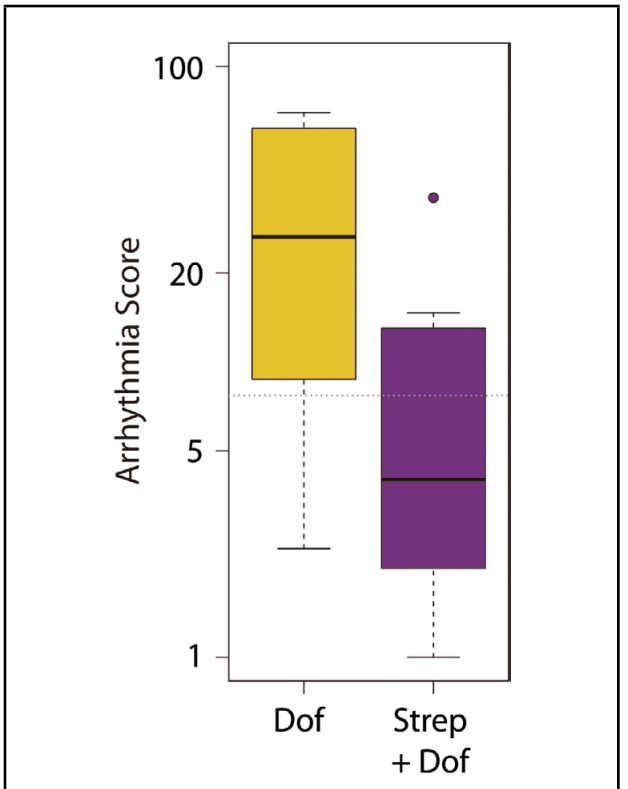

Figure 6. Preventive effect of streptomycin against dofetilide-induced arrhythmia, quantified as arrythmia score, in chronic AV-block (CAVB) dogs (CAVB; n=9), compared with CAVB dogs that were administered dofetilide alone (n=7; **Figure 3A**). Streptomycin showed a strong trend towards an antiarrhythmic effect (P=0.07 vs. control, Wilcoxon rank-sum test).

process, requiring local synchronization of EADs before an ectopic beat is induced (source-sink mismatch).[34] Well-timed stretch during late repolarization might provide this synchronizing inward current that triggers ectopic beats in the intact heart. It has been shown that a static and non-excessive volume increase results in shortening of the action potential near the plateau level, whereas at the end of the action potential it can prolong, with an 'afterdepolarization'-like prolongation.[22] This depolarization can result in an afterdepolarization-like event, but can also result in triggering of an ectopic beat.[35] Kim et al showed that in rats, either a sudden increase or reduction of stretch could trigger ectopic beats. This sensitivity to stretch-induced ectopy was increased after hypertrophy, and could be suppressed with streptomycin.[36] In the CAVB dog, increased stretch combined with a negative electromechanical window mentioned earlier, might promote formation of EADs and induction of TdP.[22,37]

No Effect of PQ Variability on Arrhythmogenesis

As shown before and here also, STV_{MAPD} is an electrical biomarker for proarrhythmia that can be measured under certain conditions, here as a result of preload variation, and represents a surrogate marker for the level of repolarization reserve. As contra intuitive as it appears, biomarkers may or may not be involved themselves in the biological processes they predict. We did not observe a difference in arrhythmia severity after dofetilide, between the two serial experiments with constant PQ or alternating PQ (ventilation on); under both conditions, ectopic beats and TdP episodes were induced in most dogs (**Figure 3C**). In other words, the decrease in repolarization reserve itself is not caused by pacing-induced preload variation.

However, because we can no longer control preload variability after onset of ventricular ectopy for obvious reasons, a proarrhythmic role of preload variation in the progression from ectopic beats to TdP cannot be excluded.

We can use the analogy of a canoe and a large trawler on a flat sea with 1 sailor in it. When the sailor will not move (=constant preload), both vessels will not rock (=low and equal STV), although we know the canoe is less stable (compromised repolarization reserve) than the trawler. When the sailor will move from left to right in the vessels (=alternating preload), the canoe will rock strongly (=large increase in STV), whereas the trawler will rock only minimally (=small increase in STV). From this, it is clear that a trigger (moving sailor or preload variability) that acts to disturb the equilibrium will demonstrate the stability of the system (boat or electrical repolarization). During a typhoon on a rough sea (dofetilide challenge), the canoe will collect relatively more water (=single and multiple ectopic beats), and eventually sink (=TdP), compared to the much more stable and larger trawler. Obviously, it will not matter much whether the sailor moves from left to right (presence or absence of preload variation) in his vessel under these conditions.

Clinical Applications of BVR for the Evaluation of Arrhythmic Risk

Simple, non-invasive actions during ECG recording can be used in arrhythmia risk prediction.[38] Our data confirm the usefulness of STV_{MAPD} for arrhythmic risk prediction, but with the important finding that a controlled challenge is required. This opens perspective to apply changes in preload to increase the predictive value of STV of repolarization for arrhythmias. In patients with an implanted pacemaker, a dedicated pacing protocol provoking changes in preload, while monitoring electrograms, may be useful for continuous monitoring of arrhythmic risk. Currently, patients undergoing cardiac resynchronisation therapy are being studied. Their instrumentation enables pacing protocols during electrophysiological examination, which result in preload variability, and subsequent EGM or ECG recordings allows STV analysis.

Study Limitations

Although in our study the pacing-induced variation in PQ was similar in AAVB and CAVB, we cannot rule out that hemodynamic or structural changes at CAVB play a role in the altered response of repolarization. For instance, the diastolic inner diameter of the left ventricle is increased in CAVB, changing the relation between ventricular pressure and the stretch the individual myocytes are subjected to.[39] Therefore, the definition of an equivalent variation in preload is not straightforward and factors other than repolarization reserve may play a role in the increased response of LV MAPD to PQ variation after remodeling. More specific blockers are required to confirm a role of the SAC in TdP induction.

Conclusions

In the anesthetized AV-block dog, the underlying mechanism of BVR is an augmented response of repolarization to beat-to-beat changes in cardiac preload when repolarization reserve is reduced; the increase in BVR after proarrhythmic remodeling and just before TdP induction with dofetilide depends on beat-to-beat variability of preload. Mechano-electrical feedback through the SAC appears to be involved in BVR. For the use of BVR as an arrhythmic biomarker, applying controlled changes in preload may be a safe way to improve predictive value in a clinical setting.

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Conflicts of Interest

None.

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Supplementary Files

Supplementary File 1

Methods

Please find supplementary file(s);
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