Absence of reverse electrical remodeling during regression of volume overload hypertrophy in canine ventricles

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Abstract

Objective: Ventricular hypertrophy predisposes for cardiac arrhythmias, presumably due to prolongation of repolarization (electrical remodeling). The temporal relation between the development of hypertrophy and electrical remodeling, as well as their reversibility upon restoration of normal load, are poorly understood. This was investigated in the present study using volume overload hypertrophy induced by atrio-ventricular (AV) block and normalization of load by pacing. Methods: Dogs were subjected to either 16 weeks of AV-block (CAVB group, n=9) or 8 weeks of AV-block followed by 8 weeks of right ventricular (RV) pacing at physiological heart rate (CAVB+PACE group, n=9). Results: Left ventricular (LV) mass (2D-echocardiography) increased after 8 weeks of AV-block to 30% above baseline and returned to 14% after 8 weeks of pacing. QT-time (surface ECG) also increased after AV-block. However, 8 weeks of pacing did not decrease QT and QTc-time (corrected for heart rate), neither during physiological pacing nor during temporary pacing at 100 beats/min. Lack of reverse electrical remodeling was confirmed by the absence of changes in LV and RV action potential duration (monophasic action potentials) at week 8 and 16. Conclusions: In volume overload hypertrophy due to AV-block, structural and electrical remodeling develop in parallel but restoration of physiological heart rate causes dissociation between reverse structural remodeling and reverse electrical remodeling.

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1. Introduction

Cardiac hypertrophy is an adaptive response of the heart to chronically increased workload. Ventricular hypertrophy significantly increases the risk of ventricular arrhythmias and sudden (arrhythmic) death, even in the absence of heart failure [1–4]. The arrhythmias in hypertrophic hearts seem to result from prolonged repolarization (for review see Ref. [5]).

These and other studies [4,6–8] showed an association between hypertrophy and prolonged repolarization, but did not investigate their temporal relationship. Our group has recently shown that hypertrophy and prolongation of QT-time seem to develop along a similar time path during the first weeks of volume overload [9]. Reversibility of prolongation of repolarization during regression of hypertrophy may also be of clinical importance, because this may predict whether treatment of hypertrophy can also be expected to reduce the risk for arrhythmias. In experimental [6,10] and clinical studies [7,8,11] (partial) regression of pressure overload hypertrophy is accompanied by reduction of prolongation of repolarization. However, in a model of age-related hypertrophy, electrical remodeling...
appeared to be irreversible [12]. Interestingly, the degree of prolongation of repolarization in the latter study was greater than that in the studies on pressure overload hypertrophy, but similar to the prolongation observed in bradycardia-induced volume overload hypertrophy, induced by chronic complete atrio-ventricular (AV) block [4,9].

In the present study we investigated the development of hypertrophy and electrical remodeling in time, as well as their reversibility using serial measurements in dogs with chronic AV-block. We considered the AV-block model highly suitable for a study on the relation between structural and electrical remodeling for the following reasons: (1) the relatively pronounced prolongation of repolarization is related to increased risk for arrhythmias; (2) AV-block is easy to create and; (3) it results in compensated hypertrophy with little loss of animals. Moreover, the increased volume load during AV-block can be normalized by using pacing at the physiological heart rate, so without the use of pharmacological agents, which might affect repolarization through other means than through regression of hypertrophy.

2. Methods

Animal handling was performed according to the Dutch Law on Animal Experimentation (WOD) and the European Directive for the Protection of Vertebrate Animals used for experimental and other purposes (86/609/EU). The protocol was approved by the Animal Experimental Committee of the Maastricht University. The experiments were performed on 18 adult mongrel dogs of either sex, body weight 28±5 kg (mean±S.D.).

2.1. Experimental protocol

After pentothal induction, anesthesia was maintained by ventilation with oxygen and nitrous oxide (1:2) in combination with infusion of midazolam (0.1 mg/kg/h, i.v.) and sufentanil (3 µg/kg/h, i.v.). Complete AV-block was created by RF ablation [13]. Prior to AV-block, a pacemaker (Medtronic Synergist H7027, H7071, Elite II or Thera DR 7941) was implanted subcutaneously in the neck. Transvenous leads (Bakken Research Center, Medtronic, Maastricht, the Netherlands) were positioned in the right atrium and right ventricular (RV) apex. The ventricular (4057M 65 cm) and atrial leads (Capsure 4503M 53 cm) were guided subcutaneously towards the pacemaker-pocket. The animals received ampicillin before (1000 mg, i.v.) and after (1000 mg, i.m.) surgery.

Subsequently, dogs were randomly divided into two groups: 16 weeks of AV-block (CAVB, n=9) and 8 weeks of AV-block followed by 8 weeks of ventricular pacing (CAVB+PACE, n=9). During the period of AV-block, the pacemaker was programmed at VVI 30 in order to prevent heart rate to fall below 30 beats/min. In the CAVB+PACE group, the pacemaker was reprogrammed after 8 weeks of AV-block to the VDD-mode (atrial sensing and ventricular pacing with 100 ms AV-delay) to restore physiological heart rate.

2.2. Echocardiography

Echocardiographic recordings were made before and bi-weekly after creation of AV-block. The animals were premedicated with acepromazine (0.6 mg/kg, i.m.) and sedated with propofol (0.4 mg/kg, i.v.). Two-dimensional echocardiographic images of the left ventricle (LV) were made and were recorded on videotape [14]. Parasternal cross-sectional images were made at the mid-papillary level (Fig. 1). QT-time was measured in lead II of simultaneously recorded ECGs.

2.3. Monophasic action potentials

Monophasic action potentials (MAPs) were measured at week 4 and 16 (CAVB group) or at week 8 and 16 (CAVB+PACE group). The different time-points for the first MAP study between the groups was chosen in order to obtain more information on the time-course of action potential duration (APD) changes after induction of AV-block without too many interventions. The anesthetic regime was similar to the AV-block procedure, LV and RV endocardial MAPs were recorded simultaneously using quadripolar contact electrodes [4]. The RV MAP catheter was placed minimally 3 cm away from the RV pacing site. The experiment was started when MAPs were stable, had a constant configuration and slope and amplitude of at least 15 mV [15]. Measurements were recorded after a 3 min stabilizing period, during idioventricular rhythm (IVR), during VDD-pacing and during fixed rate ventricular pacing at 100 beats/min. Due to irregular heart rate or poor quality of the MAPs, one dog was excluded in the CAVB and three in the CAVB+PACE group.

2.4. Terminal procedure

After the last MAP-measurements at 16 weeks, the thorax was opened and the heart was arrested with ice-cold KCl. The heart was quickly removed and both ventricles were weighed. For histological analysis a transmural section of the LV free wall was taken, immersion fixed in 10% zinc-buffered formalin and embedded in paraffin.

2.5. Echocardiographic analysis

Global LV dimensions were measured as described previously [14]. In short, for each time-point end-diastolic video images of three heartbeats were digitized off-line. The images were analyzed after blinding the images for the observer. The endocardium, epicardium and papillary
Fig. 1. Examples of LV echocardiograms in sinus rhythm (SR, panel A), after 8 weeks of AV-block (panel B), and after 8 weeks of AV-block followed by 8 weeks of pacing (panel C). Panel D displays the time-course of LV mass in this dog, expressed as a fraction of $t=0$.

muscle contours were marked manually. Inner and outer radii were determined by fitting the original epicardial and endocardial contourpoints [14]. LV cavity and wall volume were calculated using cylinder-ellipsoid model calculations. A linear relation was found between echocardiographically determined LV mass and LV weight, assessed post mortem, with $y=1.08x+6.4$ ($R^2=0.78$).

2.6. Map analysis

Applying a custom made computer program (ECG-view, Maastricht University) with a resolution of 2 ms and adjustable gain and time scale, the following parameters were measured: cycle length of the idioventricular or paced rhythm, LV and RV APD at 90% repolarization. All electrophysiological data reported are the mean of five consecutive beats. QT-time and APD were corrected for heart rate according to van de Water (QT, and APD, respectively) [16], a method that corrects QT and APD accurately under a wide range of frequencies.

2.7. Histological analysis

Morphometry was performed with a Quantimed 570 image analyzer (Leica) [14]. Myocyte width was determined using a modification of the Azan technique and collagen fraction was determined using Sirius Red staining. The data for myocyte width and collagen fraction were compared with data from dogs in sinus rhythm used in a previous study in our laboratory [14].

2.8. Statistical analysis

For group comparison ANOVA was used. ANOVA for repeated measurements was used to evaluate changes in echocardiographic and electrophysiologic variables during the course of the experiment. If significant differences were found, significant points were isolated using a Bonferroni multiple comparison test. Data are presented as mean values±S.D. $P<0.05$ was considered significant.
3. Results

In all dogs of the CAVB+PACE group, cardiac pacing was successfully applied. One CAVB dog showed temporary signs of failure (ascites) and received furosemide 5 mg/kg orally for 5 days; one CAVB dog had ascites at sacrifice.

3.1. Serial changes of LV mass

The time-course of changes in LV mass of one dog in the CAVB+PACE group is illustrated in Fig. 1, the average time-course of LV mass in both groups in Fig. 2. Both groups of dogs showed approximately 30% LV hypertrophy after 8 weeks of AV-block (CAVB 27±14%, CAVB+PACE 29±19%). In the CAVB group LV mass did not increase beyond the value reached at week 8 (maximum increase 33±11% at week 16). In the CAVB+PACE group pacing caused a rapid reduction of LV hypertrophy in the first 2 weeks, followed by a more gradual reduction till 10±14% above baseline (not significantly different from baseline LV mass) after 8 weeks of pacing. During pacing, the difference between the CAVB and CAVB+PACE group became significant from week 14 on.

Changes in LV cavity volume were qualitatively similar to those in LV wall volume, but more pronounced. As a consequence, the ratio of LV cavity volume to wall volume increased significantly from 0.82±0.07 to 0.90±0.10 after 8 weeks of AV-block and decreased to 0.75±0.07 after 8 weeks of pacing.

3.2. Post mortem observations

Post mortem data from CAVB and CAVB+PACE animals were compared with those of dogs with normal sinus rhythm (SR-group, n=14) from virtually concurrent studies [4,14]. Total heart weight to body weight (H/BW) ratio was significantly larger in the CAVB group (12.5±1.6 g/kg) than in the SR-group (7.7±1.2 g/kg). H/BW in the CAVB+PACE group (8.9±1.6 g/kg) was not significantly different from the H/BW in the SR-group, but significantly smaller than the H/BW in the CAVB group. Similarly, LV/BW and RV/BW (Fig. 3) were significantly larger in CAVB dogs (7.1±1.0 and 2.6±0.5 g/kg, respectively) than in SR dogs (4.5±0.9 and 1.6±0.5 g/kg, respectively) and in CAVB+PACE dogs (5.0±0.9 and 1.9±0.4 g/kg, respectively). Myocyte width and collagen fraction were not significantly different between the three groups (Fig. 3).

3.3. Repolarization parameters

Acutely after AV-block QT-time increased slightly (Fig. 4) due to bradycardia, because QTc-time did not increase (Table 1). A significant increase in QT and QTc-time occurred during chronic AV-block (electrical remodeling). QTc-time increased after induction of AV-block by approximately 20% within 2 weeks (Fig. 5) and did not change significantly till 8 (18±14% above baseline) and till 16 weeks (CAVB, 23±20% above baseline). Sustained prolonged repolarization was confirmed by the similar APDc values at 4 and 16 weeks in both LV and RV (Fig. 6A).

After 8 weeks of pacing in the CAVB+PACE group QTc-time remained prolonged (21±5% above baseline at week 16) despite the regression of LV mass (Fig. 5). Also, during temporary pacing at 100 beats/min absolute QT-time and JT-time (=QT-time −QRS duration) were not significantly different between week 8 and 16. The absence of reduction in prolongation of repolarization was confirmed by APDc measurements, which showed similar values of both LV and RV at week 8 and 16 (Fig. 6B). The data presented in Fig. 6 were obtained during IVR in the CAVB group and during VDD-pacing in the CAVB+PACE group, but similar values were found during temporary VDD-pacing in the CAVB group and temporary IVR in the CAVB+PACE group. No significant relation was observed between changes in LV mass and LV APDc between week 8 and 16 ($r^2=0.24$, Fig. 7). Interventricular dispersion (LV−RV APDc) was not significantly different between week 4 and 16 in the CAVB group (36±20 and 49±25 ms, respectively) or between week 8 and 16 in the CAVB+PACE group (32±38 and 25±16 ms, respectively).

4. Discussion

The present study in dogs demonstrates that after 8 weeks of AV-block restoration of physiological heart rate by ventricular pacing leads to almost complete regression of biventricular hypertrophy. This regression, however, is not accompanied by reversal of electrical remodeling: repolarization remains prolonged after up to 8 weeks of...
Fig. 3. Parameters of hypertrophy in three groups of dogs: sinus rhythm (SR, open bars), CAVB (striped bars) and CAVB+PACE (hatched bars). *P<0.05 vs. SR, ‡P<0.05 vs. CAVB.

Fig. 4. Example of changes in QT-time in one dog due to the creation of AV-block. QT-time increased from 215 ms in SR to 275 ms in acute AV-block. After 6 weeks of AV-block, QT-time stabilized at 415 ms. Note changes in QRS-morphology, which contribute to but are not the cause of the QT prolongation.

Table 1
Electrophysiological adaptations after chronic AV-block

<table>
<thead>
<tr>
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<th>SR</th>
<th>Acute AV-block</th>
<th>Chronic AV-block</th>
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<tbody>
<tr>
<td>RR interval (ms)</td>
<td>868±175</td>
<td>1242±243*</td>
<td>1334±219*</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>293±36</td>
<td>313±37*</td>
<td>378±43*</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>304±23</td>
<td>292±33</td>
<td>349±32*</td>
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Electrophysiological parameters were determined in a subset of dogs from both groups at sinus rhythm (SR) and during acute and chronic (4–8 weeks) AV-block (n=8). *P<0.05 vs. SR, †P<0.05 vs. acute AV-block.

In previous studies, the phenotype of the dog with chronic AV-block has been characterized extensively [4,17–20]. The bradycardia-induced volume overload leads pacing despite regression of hypertrophy within 2 weeks of pacing.

4.1. AV-block dog

In previous studies, the phenotype of the dog with chronic AV-block has been characterized extensively [4,17–20]. The bradycardia-induced volume overload leads
to a number of contractile, structural and electrical adaptations. Hemodynamically, the dog has no signs of heart failure at the slow heart rate [19] due to a clear increase in inotropism. This is in part based on an increased Na\(^+\)/Ca\(^{2+}\) exchanger current and increased Ca\(^{2+}\) storage in the sarcoplasmic reticulum, leading to an increased Ca\(^{2+}\)-transient [20].

During chronic AV-block prolongation of repolarization develops within 2 weeks (Fig. 5) and was confirmed by action potential duration measurements using micro-electrode techniques in isolated myocytes [17]. Patch clamp studies in myocytes revealed a decrease in \(I_{\text{c}}\)-currents but not in other potassium currents [18]. Based on the absence of changes in QRS duration between acute and chronic AV-block, we conclude that there are no changes in gap-junctions during chronic AV-block.

4.2. Structural adaptations: time dependency and reversibility

AV-block leads to rapid development of LV hypertrophy, which levels off after 4–6 weeks and remains constant till week 16. Regression follows a similar pathway: a rapid decrease in LV mass after 2 weeks of pacing followed by a more gradual reduction up to 8 weeks of pacing. Post mortem measurements also show complete regression of RV hypertrophy. The similar collagen fraction in the two groups indicate that the amount of collagen varied in proportion to changes in myocyte volume.

The faster regression of LV mass in the present study (2 weeks) than in other experimental studies (3–6 months) [6,10,21] could be explained by the shorter duration of hypertrophy in the present study (8 weeks vs. 3–6 months [6,10,21]). Longer duration of hypertrophy and a larger degree of hypertrophy (~65%) may also explain the slower regression of hypertrophy in clinical studies (6 months to 5 years) [7,8,11,22–24] than in the present study.

4.3. Electrophysiological adaptations: time dependency and absence of reversibility

After creation of AV-block electrical and structural remodeling seem to develop in parallel with each other, electrical remodeling appearing slightly quicker than hypertrophy (compare Figs. 2 and 5). In contrast, regression of hypertrophy is not associated with any reduction of prolonged repolarization. This is the case for the LV, where electrical remodeling is more pronounced than in the RV [4,19] as well as for the RV, where hypertrophy is more pronounced than in the LV. The lack of change of the repolarization parameters is independent of heart rate and activation sequence because the measurements during idioventricular rhythm, VDD-pacing, and pacing at 100 beats/min showed the same results. The fact that our measurements of repolarization were performed serially and that there is no correlation between the changes in

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**Fig. 6.** Action potential duration, corrected for heart rate (APD\(_c\)) in LV and RV at 4 and 16 weeks of AV block (CAVB group, panel A) and after 8 weeks of AV-block and 8 weeks of pacing (CAVB+PACE-group, panel B). CAVB dogs were measured during IVR and CAVB+PACE dogs during pacing. White bars depict the first measurement, the black bars the last measurement.
relative change of LV mass and LV APD (Fig. 7) supports the idea that regression of hypertrophy occurs independently of electrical changes. It is unlikely that the lack of electrical remodeling is due to a maintained elevated level of mechanical load. After all, the ratio of LV cavity volume to wall volume, closely related to wall stress and strain [25], decreases to baseline levels after 8 weeks of pacing.

Apparently processes responsible for absence of reverse electrical remodeling, like reversal of the enhanced activity of the Na⁺/Ca²⁺-exchanger and reversal of reduced Iₖ current, occur considerably slower than processes responsible for reverse structural remodeling. The unchanged collagen fraction suggests that the extracellular matrix does not play a role in electrical remodeling in the chronic AV-block model.

The absence of reverse electrical remodeling in volume overload hypertrophied hearts is in contrast with findings in pressure overload hypertrophied hearts [6–8,10,11,26]. In one of these experimental studies the hypertrophy was related to overactivation of the renin–angiotensin system and was regressed using ACE inhibitors and AT1-blockers [6], whereas in another study hypertrophy due to increased aortic outflow resistance regressed after surgical repair [10].

Reverse electrical remodeling in clinical studies was associated with regression of pressure overload hypertrophy due to treatment with ACE inhibitors or AT1-blockers [7,8,26] or due to surgical repair [11]. The fact that repolarization times decrease could be due to the reduced prolongation of repolarization (10%) [7,8].

Beside the present study, only one other study showed lack of electrical remodeling during regression of hypertrophy [12]. In that study treatment of 30-month-old non-hypertensive rats with ACE-inhibitors caused regression of hypertrophy, but had no effect on QTₜ-time. A common feature in our and Kreher’s [12] study is that the increase in QTₜ-time is more (20–30%) than in the studies sharing reduced prolongation of repolarization (7–16%) [6,10].

Reversibility of electrical remodeling may, therefore, depend on the severity of electrical remodeling and/or on the kind of hypertrophy, i.e. more reversible in pressure than in volume overload hypertrophy.

4.4. Experimental limitations

A potentially confounding factor for the measurement of repolarization parameters is the abnormal sequence of electrical activation of the ventricles, especially during pacing. However, we have previously shown that chronic ventricular pacing in non-hypertrophied [14] and pressure overload-hypertrophied dog hearts [27] has no effect on QTₜ-time. Therefore, T-wave variability due to pacing can be excluded as a confounding factor in the analysis. This is further supported by the unchanged APDₜ values, which, after all, were measured locally.

It is unlikely that the conclusions on the absence of reverse electrical remodeling after 8 weeks of pacing are flawed by the fact that APDs were not measured at week 0 or that the timing of the first APD measurement in both groups was different (week 4 in the CAVB and week 8 in the CAVB+PACE group). Intergroup comparison showed no significant difference in APD. Moreover, serial QT-time measurements reveal no change between 4 and 8 weeks, both in the CAVB and in the CAVB+PACE group.

5. Conclusions

The findings in the present study show that after the creation of AV-block electrical remodeling develops slightly quicker than structural remodeling. Restoration of physiological heart rate, and thereby normalization of volume load, causes dissociation between reverse structural (rapid and almost complete regression of hypertrophy) and electrical remodeling (no reversion of prolonged repolarization).

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