Contractile Adaptations Preserving Cardiac Output Predispose the Hypertrophied Canine Heart to Delayed Afterdepolarization–Dependent Ventricular Arrhythmias

S.H. Marieke de Groot, MD, PhD; Marieke Schoenmakers, MD; Mirella M.C. Molenschot, MD; Jet D.M. Leunissen; Hein J.J. Wellens, MD, PhD; Marc A. Vos, PhD

Background—In dogs, chronic complete atrioventricular block (CAVB) results in structural (biventricular hypertrophy) and electrical (delayed repolarization) remodeling, which predisposes the heart to torsade de pointes arrhythmias. We assessed the contractile alterations in the CAVB dog and tested the hypothesis that these adaptations increase delayed afterdepolarization (DAD)–dependent triggered arrhythmias.

Methods and Results—Steady-state and dynamic (fast pacing: 1 to 68 stimuli) left and right ventricular systolic and diastolic parameters were determined by positive and negative inotropic interventions at acute AVB and CAVB. Concomitantly, left and right ventricular endocardial monophasic action potentials were registered. In CAVB, all systolic contractile parameters were markedly increased, resulting in preserved cardiac output. The increase was most pronounced at low heart rates, altering the force-frequency response. At both acute AVB and CAVB, the degree of potentiation of cardiac function with pacing was dependent on the number of stimuli and showed a maximum at 8 to 13 stimuli. With CAVB, this potentiation curve was shifted upward, and it was only then that pacing resulted in DADs (in 8 of 10 dogs) and ectopic beats (EBs, in 6 of 10 dogs). The incidence of EBs in relation to the number of stimuli also had a maximum at 8 to 13 stimuli. Ouabain increased the incidence of DADs and EBs, whereas the negative inotropic interventions prevented them completely.

Conclusions—The alterations responsible for improvement in systolic contractile function in CAVB dogs predispose the hypertrophied heart to DAD-dependent triggered arrhythmias during positive inotropic interventions. (Circulation. 2000;102:2145-2151.)

Key Words: tachycardia n hypertrophy n contractility n electrophysiology

Delayed afterdepolarization (DAD)–induced triggered arrhythmias (TAs) have been described under conditions of intracellular calcium overload. Positive inotropic interventions favor their initiation. In anesthetized dogs with chronic complete AV block (CAVB), DAD-dependent ectopic beats (EBs) and ventricular tachycardia (VT) have been observed after the combination of ouabain and pacing. Also, we have described that CAVB is associated with electrical and structural remodeling, eg, prolonged repolarization times and biventricular hypertrophy. In this study, we tested the hypothesis that contractile alterations increase arrhythmogenic potential in this dog, especially in regard to DAD-dependent TAs. Therefore, using positive and negative inotropic interventions, we related the occurrence of TAs with left and right ventricular (LV and RV) contractile function of the dog in sinus rhythm (SR) directly after AV block (AAVB) and in CAVB under both steady-state and dynamic circumstances. In the accompanying study, the cellular changes underlying these contractile adaptations were assessed.

Methods

Under aseptic conditions, we performed 50 experiments in 34 anesthetized mongrel dogs of either sex (body weight 27.5±5 kg). All experiments were performed in accordance with the European directive for the protection of vertebrate animals used for experimental and other scientific purposes. The dogs were tested serially: in SR, at AAVB, and at 6 weeks of CAVB in 3 groups: (1) LV and RV steady-state hemodynamic studies were followed by force-frequency (FF) and cardiac output (CO) determinations, (2) LV and RV postextrasystolic potentiation (PESP) and poststimulus potentiation (PSP) assessment, and (3) determination of the relation between the inducibility of EBs to LV PSP with negative and positive inotropic interventions. Because of technical difficulties and sudden cardiac death, dogs were added to increase the number with CAVB. For the procedures to induce anesthesia, to create AVB, to place LV electrodes, and for perioperative measures, we refer to our previous publication. A 6-channel ECG was recorded. Under fluoroscopic guidance, catheters were introduced to register LV and RV monophasic action potentials (MAPs) and pressure curves. CO was determined by thermodilution in the pulmonary artery. All signals were stored at 1 kHz.
Pacing Protocols, Interventions, and Data Analysis
At AAVB and CAVB, the FF protocols (3 minutes of steady state) consisted of cycle lengths (CLs) of 300, 575±25 (equal to SR), 750, 1000, and 1250 ms. The PESP protocol consisted of a basic rhythm of 600 ms, which was interrupted every 20 beats by extrastimuli with decreasing coupling intervals from 550 to 250 ms. The PSP trains were delivered with an interstimulus interval of 300 ms and 1 to 68 stimuli. Three interventions were performed, after which PSP was repeated: at AAVB and CAVB, (1) 20 μg/kg ouabain IV was given (n=6) and (2) fixed-rate pacing (FRP) with the SR CL (520±40 ms, n=5) was performed with constant recovery interval. The third intervention, ryanodine (10 μg·kg⁻¹·10 min⁻¹), was administered only to 4 DAD-susceptible CAVB dogs. Pacing was performed from either the LV electrode (FF and PESP) or the RV MAP (PSP). By use of a software program, data were analyzed offline: LV and RV end-systolic pressure (ESP), end-diastolic pressure (EDP), and dP/dt max. From the ECG, CL idioventricular rhythm, CL PP interval, and QT time were determined. To correlate the functional adaptations with TA, we measured (1) coupling interval of the first beat postpacing, (2) LV and RV action potential duration of the MAP at 10% repolarization (MAPD), and (3) LV and RV +dP/dt before each pacing train and of beats 1 to 3 postpacing, either spontaneous, ectopic, or paced. DADs were defined as the MAP as an afterpotential with a diastolic slope of ≥10 mV/s. EBs were defined as ventricular activations with a postpacing interval <600 ms. VT was defined as ≥5 consecutive EBs. We refer to inducible dogs as those that responded with either EBs or VT.

Heart Weight
We confirmed that the CAVB dogs had (biventricular) hypertrophy: the ratio of heart to body weight was 11.5±2.1 g/kg. Slicing the heart in a subset of dogs (n=6) revealed 5.8±1.2 and 2.4±0.3 g/kg for the LV and RV, respectively.

Statistics
Data are presented as mean±SD unless otherwise stated. Statistical tests included (P<0.05) repeated ANOVA followed by Bonferroni’s t test, 2-tailed Student’s t test for unpaired events, χ² testing, and logarithmic regression analysis.

Results
Despite an increase in stroke volume, CO was reduced (P<0.05) when hemodynamics at AAVB were compared with SR (Table 1). Both ventricles show an increase in EDP with comparable systolic function (ESP and +dP/dt max). After 6 weeks of CAVB, the LV and RV systolic parameters are significantly increased (+dP/dt: +100%), resulting in an enhanced stroke volume compared with SR and return of the CO and EDP values. Steady-state pacing at slow rates showed similar increases for LV and RV +dP/dt between AAVB and CAVB (Figure 1, top). Increasing the rate resulted in a positive inotropic response at AAVB. At CAVB, however, the FF response was attenuated: the LV shows a decrease, whereas the RV curve is flat.

**PESP and PSP at Baseline**
At AAVB and CAVB, the PESP protocols resulted in an increase in +dP/dt max when the extrastimulus interval was decreased. The values obtained were higher at CAVB (Figure 1, bottom). At all time points, a fast pacing train resulted in a clear potentiation of cardiac function, which disappeared within 3 to 5 beats (Table 2 and Figure 2). This PSP was dependent on the number of stimuli: +dP/dt max increased up to a maximum at ~10 beats (Figure 3) and declined with a further increase in the stimuli (for details see Figure 5). These LV and RV PSP curves were comparable between SR and AAVB but were clearly shifted upward at CAVB.

**Triggered Ventricular Arrhythmias at Baseline**
At AAVB, pacing never resulted in the induction of DADs or EBs. In contrast, at CAVB, similar pacing trains induced EBs...
in 6 of 10 (P<0.05) and VT in 1 of 10 dogs, which coincided with DADs in the MAPs in 8 of 10 dogs (P<0.05). An example is shown in Figure 2. The number of induced EBs also varied depending on the number of stimuli (Figures 4 and 5). Most EBs were induced after 5 to 13 stimuli, which corresponded with the highest postpacing LV +dP/dt.

**Inotropic Interventions Modulating Arrhythmias**

At AAVB, the positive inotropic effect of ouabain or FRP did not result in the induction of DADs or EBs (Table 3). At CAVB, in contrast, ouabain increased the induction of arrhythmias (Figures 5 and 6), which was accompanied by a sawtooth appearance in the potentiation curve (Figure 5, top right). The induction of EBs was broadened when the different numbers of stimuli were compared (bottom right), and the numbers of EBs were increased (Table 3), sometimes resulting in VT (Figure 6).

At CAVB, the negative inotropic effects of FRP (Table 3) or ryanodine (data not shown) were accompanied by prevention of all DADs and triggered EBs.

To describe the PSP curve, we determined the postpacing LV +dP/dt of any first postpacing beat, independent of coupling interval or activation sequence. Figure 7 shows PSP of single pacing trains subdivided for AAVB and CAVB and

**TABLE 2. Electrophysiological and Hemodynamic Adaptations Before and After PSP**

<table>
<thead>
<tr>
<th>SR</th>
<th>AAVB</th>
<th>CAVB</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>520±45</td>
<td>380±70*</td>
</tr>
<tr>
<td>CL IVR</td>
<td>520±45</td>
<td>1250±240*</td>
</tr>
<tr>
<td>QT</td>
<td>240±30</td>
<td>300±30*</td>
</tr>
<tr>
<td>LV MAPD</td>
<td>220±15</td>
<td>288±36*</td>
</tr>
<tr>
<td>RV MAPD</td>
<td>201±14</td>
<td>256±22*</td>
</tr>
<tr>
<td>ΔMAPD</td>
<td>±6</td>
<td>±6</td>
</tr>
<tr>
<td>CI first beat</td>
<td>585±75</td>
<td>1200±280*</td>
</tr>
</tbody>
</table>

IVR indicates idioventricular rhythm; ΔMAPD, interventricular dispersion of repolarization; Cl, coupling interval. n=11.

*P<0.05 vs SR, †P<0.05 vs AAVB, ‡P<0.05 vs LV and RV dP/dt pre, and §P<0.05 vs LV and RV dP/dt 1st beat.

**Discussion**

At 6 weeks of CAVB, steady-state LV and RV systolic contractile function is increased to such an extent that CO can be maintained. This successful adaptation remains present under positive inotropic interventions but is then accompanied by the occurrence of DADs and DAD-dependent TAs.

**The CAVB Dog: Biventricular Compensated Contractile Function**

Cardiac hypertrophy is an adaptation to mechanical overload of any cause. Whether this adaptation is able to maintain its contractile performance in time or whether there will be degeneration to congestive heart failure is still not clear. To
Quantify heart failure, numerous parameters have been developed at the integrative, (multi)cellular, and molecular level. In the nonfailing myocardium, FF and PESP are well-known phenomena that are decreased in the early stages of heart failure.10–14

In the CAVB dog, both ventricles perform adequately under baseline as well as more demanding conditions (Figures 1 and 3). The FF response in the CAVB dog is altered, however (Figure 1): increasing the rate is no longer associated with an increase in LV or RV $dP/dt_{max}$. The FF curve, however, is always increased with regard to AAVB and never reaches the data obtained in canine heart failure.12,15,16 Interpretation of this finding is complex, but rate-dependent measurements of cell shortening and calcium transients in isolated myocytes confirm that this is an intrinsic property of the cells.7 The possible role of an increased Na-Ca exchange current for improved contractile performance at slow rates is discussed in the accompanying article.7

PESP is also maintained during CAVB (Figure 1). PSP was seen during SR, AAVB, and CAVB (Figure 3), and its magnitude was dependent on the number of stimuli (Figures 3 and 5) and on the prepacing inotropic state (Figure 7). The highest PSP values were obtained at CAVB after short pacing trains consisting of 5 to 13 stimuli. To the best of our knowledge, we are the first to describe PSP in vivo. Because this curve remained present under all conditions tested, including FRP, we can exclude alterations in ventricular filling secondary to variations in the interval as the underlying mechanism. It appears that this PSP curve is intrinsic to the myocardium. All these findings are indicative of compensated contractile function in the CAVB dogs at 6 to 10 weeks.

TAs and Potentiation of Contractile Function

Electrical remodeling in the CAVB dog5,6 consists of an increase in repolarization times that exceeds the expected lengthening on the basis of rate and that is not uniform, leading to an increase in interventricular dispersion (Table 2). This dissimilar MAPD lengthening is caused by specific ventricular alterations in ionic channel function.7,17 Potential stimuli may include bradycardia, hypertrophy, and/or the

Figure 3. Effect of number of stimuli (nVs) on LV and RV PSP. Prepacing inotropic state ($dP/dt_{max}$) is depicted on y axis for SR, AAVB, and CAVB (dotted lines). Pacing induces PSP in LV (top) and RV (bottom), which for all curves reaches significance at stimulus 1 or 3 ($P<0.05$ vs prepacing $dP/dt$). Absolute values for CAVB curves are significantly higher than SR and AAVB. Increasing nVs from 1 to 35 results initially in an increase, with highest values obtained at 8 to 13 stimuli ($SP<0.05$ vs 1 stimulus).

Figure 4. Effect of number of stimuli (nVs) on induction of DAD-dependent EBs. In each panel, ECG lead II and LV MAP are shown. Idioventricular rhythm at CAVB is interrupted by pacing with 1 to 33 stimuli (S), with an interstimulus interval (Vs-Vs) of 300 ms. Arrhythmic quantification is based on diastolic slope3 and appearance of DADs and EBs. 1, Application of 1 stimulus does not lead to an EB and hardly to changes in diastolic slope of DADs (7 mV/s). 2, There is an increase in slope after application of 5 stimuli, which remains subthreshold. After 10 stimuli (3), additional increase of slope ($>20$ mV/s) is related to occurrence of 4 triggered EBs. A further increase in nVs to 13 (4) and 33 (5) stimuli results in a decreased induction of EBs and decreased slope of subthreshold DADs.
alterations in calcium homeostasis. Synergistic effects could explain why our MAPD increase exceeds those reported so far in canine LVH.18

The PESP protocols never proved to be arrhythmic. Short-lasting, fast pacing trains, however, resulted in EBs in the majority of CAVB dogs but not in those with AAVB (Tables 2 and 3). The occurrence of EBs coincided with DAD registration on the MAP (Figures 2, 4, and 6), thereby indicating triggered activity as the underlying mechanism. Curves of potentiation and of incidence of EBs were similar, suggesting a common origin. The dynamic changes in calcium handling may temporarily create an arrhythmogenic window of calcium overload in the CAVB dog, during which the DADs and EBs occur. This hypothesis was studied further by use of different interventions that modulate the inotropic state. At AAVB, 2 positive inotropic interventions (ouabain

TABLE 3. Hemodynamic and Arrhythmic Effects of Ouabain (20 μg/kg) and Fixed-Rate Pacing

<table>
<thead>
<tr>
<th></th>
<th>AAVB Control</th>
<th>Ouabain</th>
<th>IVR</th>
<th>FRP</th>
<th>AAVB Control</th>
<th>Ouabain</th>
<th>IVR</th>
<th>FRP</th>
<th>CAVB Control</th>
<th>Ouabain</th>
<th>IVR</th>
<th>FRP</th>
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</thead>
<tbody>
<tr>
<td>LV ESP</td>
<td>83 ± 12</td>
<td>88 ± 13*</td>
<td>95 ± 12</td>
<td>98 ± 14</td>
<td>102 ± 10</td>
<td>108 ± 10*</td>
<td>98 ± 14</td>
<td>99 ± 17</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LV dP/dt pre</td>
<td>825 ± 230</td>
<td>1005 ± 345*</td>
<td>915 ± 220</td>
<td>1095 ± 385</td>
<td>1685 ± 285</td>
<td>2075 ± 535*</td>
<td>1680 ± 430</td>
<td>1345 ± 325*</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>PP + LV dP/dt, 1st beat</td>
<td>1675 ± 250†</td>
<td>1795 ± 310*†</td>
<td>1715 ± 345†</td>
<td>1900 ± 515†</td>
<td>2340 ± 225†</td>
<td>2555 ± 145†</td>
<td>2370 ± 500†</td>
<td>2170 ± 500†</td>
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<tr>
<td>Arrhythmias</td>
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<td></td>
<td></td>
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<tr>
<td>Inducible dogs</td>
<td>0/6</td>
<td>0/6</td>
<td>0/5</td>
<td>0/5</td>
<td>3/6</td>
<td>4/6</td>
<td>4/5</td>
<td>0/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DAD</td>
<td>0/6</td>
<td>0/6</td>
<td>0/5</td>
<td>0/5</td>
<td>4/6</td>
<td>5/6</td>
<td>4/5</td>
<td>0/5</td>
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<td></td>
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<tr>
<td>VT</td>
<td>0/6</td>
<td>0/6</td>
<td>0/5</td>
<td>0/5</td>
<td>0/6</td>
<td>1/6</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No. of EBs</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 ± 1</td>
<td>9 ± 10</td>
<td>3 ± 1</td>
<td>0</td>
<td></td>
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*P<0.05 vs control or IVR, †P<0.05 vs LV dP/dt pre.
and FRP) did not lead to induction of any arrhythmia. The highest PSP obtained, however, was far less than the baseline CAVB value (Table 3). The same therapeutic dosage of ouabain in CAVB increased the number of pacing trains that responded with EBs, and VT even occurred (Figures 5 and 6). The negative inotropic interventions at CAVB (FRP and ryanodine) caused prevention of the induction of all DADs and EBs. The block of the sarcoplasmic reticulum calcium release channel by ryanodine has been described to abolish DAD-related EBs.19 Thus, the CAVB heart is more susceptible to DAD-dependent arrhythmias under circumstances that demand higher contractile performance, in which the increased Na-Ca exchange current can be of great importance.7 It is unclear whether this arrhythmogenic potential is related to the remodeling processes or whether it is purely due to the (maximal attained) inotropism. In the latter, individual parameters such as the basal inotropic state, maximal amount of potentiation, contractile reserve, and threshold at which the sarcoplasmic reticulum spontaneously releases calcium have to be included. Whereas the preparing inotropic state has a relation with maximal PSP and the occurrence of EBs, single-train analysis (Figure 7) did not allow prediction of the occurrence of EBs, suggesting that the inotropic state is not the single determinant.

In vitro, an increased propensity of hypertrophied myocardium to DAD-dependent TAs has been described.20,21 In these studies, stimulation alone resulted rather infrequently in DADs, whereas combining pacing with adrenergic stimulation frequently induced TAs. Because we studied intact animals, local activity of the autonomic nervous system might be related to the observed occurrence of DADs and related EBs.

Clinical Implications
Extrapolation from animal data to humans must be done with great caution. Both hypertrophy22 and heart failure23 are known to predispose the human heart to ventricular arrhythmias and sudden cardiac death. The exact mechanisms involved are not elucidated, although prolongation of the APD has been described consistently and changes in calcium handling have been implicated,23 suggesting TA as a contributing mechanism. Whether DADs occur with maximal inotropism (hypertrophy) or when the function is severely depressed (heart failure) can only be answered when combined, detailed information about the contractile performance and the arrhythmias is available and possible confounding factors, like ischemia, are excluded. This is most easily obtained in animal models. However, arrhythmogenic information has been limited to only a few studies, performed predominantly in heart failure.16,18,24,25
Limitations
Because we measured at only 2 time points, we cannot exclude a time-specific arrhythmogenic response. Moreover, AAVB and CAVB resemble 2 different neurohumoral situations that can influence the results. In AAVB, compensation mechanisms have been activated, whereas at 6 weeks of CAVB, all plasma neurohumoral parameters have returned to baseline. Second, we do not know to what extent the observed phenomena actually relate to the different remodeling processes. Other factors can also play a role in the alterations in contractile function, including the sensitivity of the cardiac contractile proteins and the interstitial tissue of the myocardium. Third, we have concentrated on the initiation of the arrhythmia by DADs, thereby excluding other mechanisms.

In conclusion, the alterations accompanying the improvement in contractile function in the CAVB dog predispose the heart to DAD-dependent TA.

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References
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