Enhanced Susceptibility for Acquired Torsade de Pointes Arrhythmias in the Dog With Chronic, Complete AV Block Is Related to Cardiac Hypertrophy and Electrical Remodeling

M.A. Vos, PhD; S.H.M. de Groot, MD, PhD; S.C. Verduyn, PhD; J. van der Zande, BSc; H.D.M. Leunissen; J.P.M. Cleutjens, PhD; M. van Bilsen, PhD; M.J.A.P. Daemen, MD; J.J. Schreuder, MD; M.A. Allessie, MD, PhD; H.J.J. Wellens, MD, PhD

Background—Chronic, complete AV block (CAVB) in the dog leads to ventricular hypertrophy, which has been described as an independent risk factor for arrhythmias. In this model, we examined (1) whether the short- and long-term electrical adaptations predispose to acquired torsades de pointes arrhythmias (TdP) and (2) the nature of the structural and functional adaptations involved.

Methods and Results—We determined (1) endocardial right (RV) and left (LV) ventricular APD, \( \Delta \text{APD} \) (LV APD–RV APD), presence of EADs at 0 weeks (acute: AAVB), and CAVB (6 weeks) and inducibility of TdP by pacing and \( d \)-sotalol (n = 10); (2) steady-state and dynamic LV hemodynamics at 0 and 6 weeks (n = 6); (3) plasma neurohumoral levels in time (n = 7); (4) structural parameters of the LV and RV of CAVB dogs (n = 6) compared with sinus rhythm (SR) dogs (n = 6); and (5) expression of ventricular mRNA atrial natriuretic factor (ANF) in CAVB (n = 4) and SR (n = 4) dogs. Compared with AAVB, CAVB led to nonhomogeneous prolongation of LV and RV APD and different sensitivity for \( d \)-sotalol, leading to EADs (4 of 14 versus 9 of 18, \( P < 0.05 \)), increased \( \Delta \text{APD} \) (45 ± 30 versus 125 ± 60 ms, \( P < 0.05 \)), and induction of TdP in most dogs (0% versus 60%, \( P < 0.05 \)). CAVB led to biventricular hypertrophy, whereas LV function was similar in AAVB and CAVB. The neurohumoral levels were transiently elevated. The LV and RV collagen and the capillary/fiber ratio remained normal, whereas ventricular ANF mRNA was not detectable.

Conclusions—The electrical remodeling occurring after CAVB predisposes the heart to acquired TdP, whereas the structural changes (hypertrophy) are successfully aimed at maintaining cardiac function. (Circulation. 1998;98:1125-1135.)

Key Words: cardiac function ■ electrophysiology ■ early after dipolarizations ■ fibrosis ■ action potentials
Hypertrophy, Electrical Remodeling, and TdP

TdP. Second, we examined the nature of the volume overload–induced hypertrophy by determining the structural and functional adaptations. The cellular basis for these changes is discussed in a companion article.18

Methods

All experiments were performed in accordance with the Guiding Principles in the Care and Use of Animals as approved by the American Physiologifcal Society (NIH publication 86–23, revised 1996) and under the regulations of the Committee for Experiments on Animals of Maastricht University. All experiments were performed on anesthetized mongrel dogs under aseptic conditions. Dog selection was based on size (body weight between 20 and 40 kg) and age (adult) but not sex. A total of 25 CAVB dogs were tested and compared with 21 dogs with normal conducted sinus rhythm (SR).

Different Groups

Duration of CAVB at the Moment of the Experiments in the

A total of 10 animals were instrumented to investigate the response with the QRS complexes, with a pulse width of 2 ms and a stimulus electrode with a custom-built programmable stimulator (Maastricht University) that delivers unipolar, rectangular stimuli synchronous with the CL of SR and (2) at a paced CL identical to the CL at AAVB.

TdP Induction Protocol

A detailed description of the TdP induction protocol, performed during a stable IVR, is described elsewhere.3 Two different pacing modes were used before and after d-sotalol (2 mg · kg⁻¹ · min⁻¹ IV). A TdP arrhythmia was defined as a polymorphic ventricular tachycardia consisting of ≥5 beats that twisted around the baseline and occurring in the presence of a prolonged QT(U) duration. A dog was called inducible when TdP could be induced ≥3 times with the same pacing mode. Variables of ventricular repolarization are heart rate–dependent, and it was previously reported that inducible dogs have a longer cycle length (CL) of the IVR than noninducible dogs.3 Therefore, in those dogs studied twice (n = 5), we performed the TdP induction protocol after d-sotalol at 2 basic CLs: (1) during spontaneous IVR and (2) at a paced CL identical to the CL at AAVB.

Serial LV Hemodynamic Measurements

For determination of the LV functional status, a catheterization was performed in 6 dogs at 2 time intervals (SR/AAVB and 6 weeks of CAVB) through the carotid artery. With a solid-state micromameter transducer catheter (Sentron), LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), and LV +dp/dt were measured during a fixed paced CL of 600 ms. In addition, postextrastricular potentiation (PESP, absolute and relative increase in LV +dp/dt) was determined at these 2 time points by application of an extrastimulus from 550 ms down to 250 ms and vice versa. The recovery interval after the extrastimulus was set at 600 ms.39 This protocol was performed before and after a bolus of 20 µg/kg ouabain. In 3 additional dogs, a conductance catheter (7.5F, Webster Laboratories) was placed in the apex of the LV directly after creation of AV block through the carotid artery. The conductance catheter was connected to a Leycom Sigma-5DF signal conditioner processor (CardioDynamics) to measure LV volume. The catheter was assumed to be placed correctly if the signals of at least the 4 most distal segments displayed a typical LV volume tracing.39 Conductance catheter stroke volume was calibrated by thermodilution stroke volume (Swan-Ganz catheter through the jugular vein). Pressure/volume (PV) loops were determined at 2 paced CLs of 600 (comparable to the CL of SR) and 1200 ms (comparable to IVR).

Data Acquisition and Analysis

All the signals consisting of the surface ECG leads and either 2 MAP signals or the pressure signal were simultaneously registered and stored on hard disk. All signals were sampled at 1 kHz. With a custom-made computer program (ECG View) with a resolution of 2 ms and adjustable gain and time scale, the following parameters were measured off-line: CL, IVR, QT time (lead II), LV and RV APD, and LV +dp/dt. All the data reported are the mean of 5 consecutive beats. In the serial TdP comparison, QT was subdivided into QTpeak and QTend. ∆APD was defined as LV APD–RV APD. Measurements were checked by an independent observer.

Serial Plasma Neurohumoral Data

Venous blood samples were taken in anesthetized dogs (n = 7) in stable hemodynamic conditions (stable capnograph for 30 minutes). Two control measurements were taken with a 1-week delay before AV block. Thereafter, samples were collected at 1, 2, 4, 6, and 8 weeks of CAVB. Blood (15 mL) was taken and distributed into 4 prechilled tubes containing EDTA, EDTA plus (with 3.6 µmol/L enalaprilat), GH (250 IU heparin and glutathione), and TE (EDTA and trisylol 50 KIU/mL blood). The blood samples were immediately centrifuged (15 minutes, 3400 rpm, 4°C), and the plasma was

No. indicates the group size in the different experiments. Total reflects the cumulative number of animals tested.
decanted, frozen in a dry ice/ethyl alcohol bath, and stored at −80°C. Angiotensin II (Ang II) was determined by radioimmunoassay after phenyl column extraction (Amersham International). Atrial natriuretic factor (ANF) was determined by radioimmunoassay (Nichols Institute Diagnostics) after Sep-Pak C18 column extraction. Aldosterone was assayed by means of a solid-phase protein binding radioimmunoassay (Diagnostic Products Corp). Noradrenaline (NE) was assessed by a sensitive fluorimetric method in which catecholamines are concentrated from plasma by liquid-liquid extraction and derivatized with a selective fluorescent agent before high-performance liquid chromatography. Most plasma assays were performed in duplicate.

**Group Comparison of the Structural Changes**

To assess the amount of hypertrophy due to CAVB, we determined heart weight (HW) in 2 groups. In the first, total HW and LV and RV weights were determined in 11 SR dogs and in 9 CAVB dogs (20±12 weeks). For this purpose, the hearts were excised, rinsed with water, and stored in 10% formaldehyde for at least 2 weeks. For ventricular weight, we isolated the ventricles from the atria and removed the RV, taking the septum as part of the LV. The measurements were corrected for differences in body weight. In a second group (6 dogs in SR and 6 dogs after 10±2 weeks of CAVB), the HW was determined but now followed by the isolation of 6 transmural specimens from 3 LV (high, mid, and apical LV free wall), 1 septal, and 2 RV (outflow and mid RV free wall) sites. These specimens were fixed in formalin and embedded in paraffin. Paraffin sections 6 μm thick were stained with Sirius red F3BA. Morphometric determinations of the collagen volume fraction in all samples (10 to 15 fields per section) were obtained by 1 investigator, while 2 independent observers partly checked the measurements.

To determine the capillary-to-fiber (C/F) ratio, 2 transmural tissue samples from the RV and LV were taken from a 5-mm-thick coronal section at the equator of the heart. The LV sample was taken from the HW was determined but now followed by the isolation of 6 transmural specimens from 3 LV (high, mid, and apical LV free wall), 1 septal, and 2 RV (outflow and mid RV free wall) sites. These samples were dehydrated in ethanol and embedded in Kulzer Technovit 8100 plastic. Cardiomyocyte and capillary basement membranes were stained on 2-μm-thick plastic sections by the Jones silver methamine method. C/F ratio was determined on 12 random fields in 1 section. Total numbers of cardiomyocytes and capillaries were counted on a 10×10 grid with border correction at a magnification of ×400.

**Group Comparison of Ventricular ANF mRNA Levels**

Total RNA was isolated with TRIzol reagent (Life Technologies) from biopsies taken from the RV and LV of 4 CAVB dogs (9.5±3 weeks), 4 dogs in SR, and 1 CAVB dog with signs of heart failure. A biopsy of the LV of 1 dog with aortic stenosis was also included. Samples of canine atrial tissue and hypertrophied rat ventricular tissue served as positive controls. DNA (10 μg) was size-fractionated on a denaturing gel with 1× MOPS running buffer. RNA was transferred to a nylon membrane (Hybond-N, Amersham) with 10×SSC (1.5 mol/L NaCl, 0.15 mol/L sodium citrate, pH 7.0) by capillary transfer and fixed by heating at 80°C for 10 minutes, followed by crosslinking under UV light (0.3 J/cm²). The filter was stained with 0.04% methylene blue in 0.5 mol/L acetic acid (pH 5.2) for 7 minutes and then destained in diethyl pyrocatecholate–treated milli-Q water until the ribosomal bands were clearly visible. A 400-bp fragment of the rat ANF cDNA was labeled with [α-32P]dCTP (3000 Ci/mmol; DuPont NEN) by random priming (Radprime, Life Technologies) to a specific activity of >0.5×10⁶ cpm/μg DNA. Filters were prehybridized for 2 hours at 58°C and hybridized (overnight) at 58°C in 6×SSC containing 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA, 0.5% SDS, and 100 μg/mL heat-denatured salmon sperm DNA. After overnight hybridization, the blots were washed and exposed to Hyperfilm MP (Amersham) with intensifying screens for 2 days at −80°C.

**Statistics**

Paired and unpaired Student’s t tests were applied to compare data obtained in the predrug condition and after d-sotalol or ouabain and between AAVB and CAVB. The χ² test was used when the data were presented as a proportion, and repeated-measures ANOVA was used to determine statistical difference for the PESP and the neurohumoral data. Values of P≤0.05 were considered significant. All data are presented as mean±SD unless otherwise stated.

**Results**

**Electrophysiological Adaptations and Induction of Acquired TdP After CAVB**

During creation of AV block, we lost 1 dog to ventricular fibrillation. In 7 of the remaining 9 AAVB dogs, the criteria with respect to the quality of both MAPs were met to ensure appropriate data sampling (7×2=14 MAPs, Table 2). Between experiments, 1 of these 7 dogs died. In another dog, we could not record adequate MAP signals during the second experiment; thus, 5 dogs could be fully analyzed at both time points. To increase the number of dogs with CAVB, we added 2 consecutive dogs that were tested only at CAVB. In total, 10 dogs were given d-sotalol, of which 9 had adequate MAPs (9×2=18 MAPs, Table 2). The data are presented as a group comparison and as a serial comparison for the 5 dogs tested twice.

**Group Comparison**

After complete AV block, a stable IVR evolved with a CL of ±1600 ms, which was maintained over time (Table 2). At

| TABLE 2. Electrophysiological Effects of CAVB and Effect of d-Sotalol |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Baseline        | d-Sotalol       |                 |                 |
|                 | 0 Weeks         | 6 Weeks         | 0 Weeks         | 6 Weeks         |
| CL of NR, ms    | 1600±280        | 1615±280        | 1720±465        | 1935±425        |
| QT, ms          | 315±25          | 390±60*         | 385±40†         | +22%            |
| LV APD, ms      | 295±20          | 390±60*         | 340±40†         | +15%            |
| RV APD, ms      | 260±20          | 315±40*         | 295±20†         | +15%            |
| ΔAPD, ms        | 40±35           | 70±30           | 45±30           | +12%            |
| EADs            | 0/14            | 1/18            | 4/14            | 9/18†           |
| TdP             | 0/9             | 1/10            | 0/9             | 6/10†           |

ΔAPD indicates LV APD–RV APD; EADs, early afterdepolarizations. Data are presented as mean±SD or as ratios. The percent increase after d-sotalol is indicated.

*P<0.05 vs 0 weeks; †P<0.05 vs baseline.
AAVB, all dogs had smooth MAP signals; no EADs (0 of 14) were present, and TdP could not be induced by pacing (0 of 9, Table 2, second column). Six weeks of CAVB led to a significant increase in all repolarization parameters (Table 2, third column). The ΔAPD augmented from 40±35 to 70±30 ms (nonsignificant, P=NS) because of the absolute and relative larger increase of the LV APD compared with the RV APD. At CAVB, 1 dog had very pronounced repolarization disorders: a relatively long QT time with a prominent U wave, EADs in the LV (1 of 18, Table 2), and a large ΔAPD (130 ms). TdP could be induced in this animal already in the absence of -sotalol (1 of 10, Table 2).

In half of the AAVB experiments, -sotalol increased the CL of IVR to such an extent that hemodynamics became compromised (fall in CO₂ values), necessitating pacing at the pre- -sotalol IVR value. Therefore, the values shown can underestimate the slowing of the IVR by -sotalol (1720±465 ms). At a comparable CL IVR, -sotalol increased the QT time and APD in both ventricles uniformly so that no effect on ΔAPD was seen (Table 2, fourth column). APDs were prolonged less after -sotalol compared with the long-term electrical effect of CAVB. The EADs were visible in 4 of 14 MAPs, but no TdP could be induced by pacing (0 of 9, Table 2). In the dogs with CAVB, the effect of -sotalol on LV APD was much more pronounced (+28% versus +15%, P<0.01, Table 2 in the fifth column) than at AAVB. EADs also developed more frequently (9 of 18, P<0.01). All these changes led to an increased ΔAPD of 125±65 ms and to the reproducible induction of TdP in 6 of 10 animals (Figure 1, bottom).

Serial Testing
When the dog was used as its own control, similar results were obtained. At AAVB, we measured QTₚₑₚₗ (260±20 ms), QTₑₚₗ (330±20 ms), RV APD (270±15 ms), and LV APD (315±35 ms). ΔAPD amounted to 45±35 ms, and the difference between QTₑₚₗ and QTₚₑₚₗ was 70±10 ms. Although -sotalol increased all individual repolarization parameters significantly: QTₚₑₚₗ (325±50 ms), QTₑₚₗ (400±50 ms), RV APD (300±20 ms), and LV APD (355±35 ms), the values for dispersion did not increase to a similar extent:
ΔAPD to 50±25 ms and QT_{end}−QT_{peak} to 75±35 ms. At AAVB, the combination of pacing and d-sotalol never resulted in TdP or ectopic beats (Figure 1). In contrast, at CAVB, d-sotalol led to significant increases in the already prolonged dispersion parameters: QT_{end}−QT_{peak} from 95±60 to 125±45 ms (P=NS) and ΔAPD from 75±35 to 120±45 ms (P<0.05). This combination resulted in TdP induction in the majority of the dogs (4 of 5, Figure 1). Compared with AAVB, TdP induction was associated with a longer APD, an increased ΔAPD (50±25 versus 120±45 ms, P<0.05), and more frequent development of EADs after d-sotalol (4 of 10 versus 8 of 10). The noninducible dog had the smallest ΔAPD and no EADs. PESP was repeatedly interrupted by ectopic beats in dogs after CAVB, whereas in AAVB, this was never the case. After correction for the somewhat slower heart rate observed in these (inducible) dogs after CAVB, pacing did not lead to a different response: the dogs remained inducible.

**Hemodynamic Evaluation of LV Function**

Under complete anesthesia, the CL of SR was 540±50 ms. Creation of AV block resulted in a CL IVR of 1320±280 ms (P<0.05), which was accompanied by an increase in LVEDP from 9±4 to 16±4 mm Hg (P<0.05), whereas LVESP remained similar (87±18 mm Hg during SR versus 91±16 mm Hg). An example of a PV loop illustrating bradycardia-induced volume overload is shown in Figure 2. At AAVB at a constant paced CL of 600 ms, the LVESP was 88±9 mm Hg, the LVEDP 11±3 mm Hg, and the LV +dP/dt 941±242 mm Hg/s. There were no differences in hemodynamic status of the LV at CAVB: 93±5, 8±4, and 1145±212 mm Hg/s, respectively. In Figure 3 (top), the PESP results are shown for the 2 time points. The absolute LV PESP response (LV +dP/dt on y axis) is shown when coupling interval (CI) of extrastimulus is decreased from 600 ms (steady state) to 250 ms (x axis). Curve at 6 weeks of AV block (AVB 6) is significantly higher ($) than at acute AV block (AVB 0), whereas potentiation is present at both times: * at different CIs indicates significantly lower value than at 250 ms. Bottom, These curves are generated by use of relative increase in LV +dP/dt, related to steady-state 600-ms values. Again, shortening of CI leads to more potentiation (*). However, curves are now identical.

**Neurohumoral Evaluation**

At 2 weeks but not at 6 weeks of CAVB, ANF (200±30 versus 540±170 pg/mL) and NE were significantly increased (P<0.05) elevated compared with control (Figure 4, left). Plasma Ang II (205±55 versus 405±65 pg/L) was also transiently in-
creased, but aldosterone did not show a significant increase (595±160 versus 900±250 pmol/L, Figure 4, right).

**Heart Weights**
Assessment of HW in dogs with CAVB (20±11 weeks) revealed a significant increase in mass compared with the SR HW (Table 3). This increase is present in the LV and RV, resulting in biventricular hypertrophy. In Figure 5, the heart of a dog in SR is compared with that of a CAVB dog of equal body weight and size (top). The LV hypertrophy seemed to be eccentric with respect to SR dogs (Figure 5, bottom). At the basal part of the RV, a clear bulging developed. The size of this cavity increased considerably, which also seemed to go along with thickening of the RV free wall. However, no definitive statements concerning volumes and wall thicknesses can be made from hearts that were not fixed at a given filling pressure or volume.

**Collagen and C/F Ratio**
In the SR dogs, all C/F ratios were well over 1 (Table 4). An example of a Jones silver methamine staining is shown in the upper part of Figure 6. The C/F ratio of CAVB dogs was smaller in 2 of the 3 comparisons: for total ventricle and for the LV. Even so, the ratios presented were well over 1. Collagen volume fraction did not differ between

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**TABLE 3.** HW of Dogs in SR Compared With Dogs With CAVB

<table>
<thead>
<tr>
<th>Weight</th>
<th>SR (n=11)</th>
<th>AV (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW, g</td>
<td>225±34</td>
<td>280±64*</td>
</tr>
<tr>
<td>H/BW, g/kg</td>
<td>7.7±1.2</td>
<td>11.2±1.9*</td>
</tr>
<tr>
<td>LV, g</td>
<td>125±26</td>
<td>145±32</td>
</tr>
<tr>
<td>RV, g</td>
<td>40±8</td>
<td>65±22*</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>4.3±0.9</td>
<td>5.8±2.0*</td>
</tr>
<tr>
<td>RV/BW, g/kg</td>
<td>1.4±0.4</td>
<td>2.6±0.9*</td>
</tr>
<tr>
<td>BW, kg</td>
<td>29.6±5.6</td>
<td>25.1±5.6</td>
</tr>
</tbody>
</table>

H/BW indicates HW/body weight. AVB was 20±12 weeks. *P<0.05 vs SR.
the 2 groups, either presented as total ventricular volume or specified to either ventricle (Table 4). An example of the Sirius red staining is shown in the lower part of Figure 6.

**TABLE 4. Structural Changes of the Heart After CAVB**

<table>
<thead>
<tr>
<th></th>
<th>SR Dogs (n=6)</th>
<th>AVB Dogs (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW, g</td>
<td>204±35</td>
<td>320±24*</td>
</tr>
<tr>
<td>BW, kg</td>
<td>26.7±5.2</td>
<td>26.7±4.3</td>
</tr>
<tr>
<td>H/BW, g/kg</td>
<td>7.7±0.6</td>
<td>12.2±1.7*</td>
</tr>
<tr>
<td>Collagen, total, %</td>
<td>2.41±0.93</td>
<td>1.97±0.98</td>
</tr>
<tr>
<td>Collagen, LV, %</td>
<td>2.18±0.84</td>
<td>1.87±0.80</td>
</tr>
<tr>
<td>Collagen, RV, %</td>
<td>2.87±0.98</td>
<td>2.16±1.30</td>
</tr>
<tr>
<td>C/F ratio, total</td>
<td>1.25±0.15</td>
<td>1.11±0.10*</td>
</tr>
<tr>
<td>C/F ratio, LV</td>
<td>1.26±0.08</td>
<td>1.13±0.09*</td>
</tr>
<tr>
<td>C/F ratio, RV</td>
<td>1.23±0.21</td>
<td>1.09±0.13</td>
</tr>
</tbody>
</table>

*BW indicates body weight. AVB was 10±2 weeks. *P<0.05 AVB dogs vs SR dogs.

**Ventricular Expression of ANF mRNA**

Figure 7 shows a Northern blot for the expression of ANF. As expected, ANF mRNA expression can be identified in the hypertrophied LV of the rat and the right atrium of the dog (control samples). Also in accordance with the literature is the absence of expression of ANF mRNA in the SR dogs. This study demonstrates that ANF mRNA was not expressed in the LV of CAVB dogs, in the heart failure dog, or in a dog with aortic stenosis (AS dog). Similar findings were obtained for the RV (left), with the exception that the heart failure CAVB dog did show a low level of expression of ANF mRNA.

**Discussion**

**Adaptive or Physiological Hypertrophy**

From clinical and experimental studies, it is well known that chronic pressure or volume overload of the heart results in myocardial adaptation (cardiac remodeling). Several canine models of ventricular hypertrophy and/or heart failure have been described that differ markedly in their presentation in regard to (1) heart (myocyte) function (compensated or decompensated); (2) level, nature, and location of myocardial growth; (3) cardiac stiffness (interstitial fibrosis); (4) energy supply (C/F ratio); (5) (re)expression of certain genes; and (6) activation of the plasma or tissue renin-angiotensin-aldo-stero- 

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**Figure 6.** Structural observations. Top, Photomicrograph of Jones silver methamine–stained plastic section for SR dog. Single cardiomyocytes are covered by silver-stained basement membrane. Capillaries are visualized by silver-stained basement membranes. These capillaries surround cardiomyocytes. Occasionally, erythrocytes are trapped in lumen of capillaries (pink anuclear cells). Scale bar=10 μm. Bottom, Photomicrograph of Sirius red–stained paraffin section for CAVB dog. Sirius red stains fibrillar collagen fibrils red. Note: no replacement fibrosis and no thickening of intercellular collagen fibrils. Scale bar=5 μm.

**Figure 7.** Expression of ANF with Northern blot. Expression of ANF mRNA is visualized for different tissues obtained in different species. As control, ventricular expression of ANF is shown for the hypertrophied rat heart (rat V). Expression of ANF can also be demonstrated in dog right atrium (dog RA). However, ANF could not be detected in either ventricle of dogs studied: RV and LV of SR dogs, LV and RV of normal CAVB dogs (AVB dogs), LV of dog with aortic stenosis (AS dog), or LV of AVB dog with evident congestive heart failure. Only in RV of this dog could expression of ANF be seen (bottom left).
dance with the eccentric hypertrophy we noticed, although small increases in wall thickness have been described. In the companion article, these findings are confirmed on the cellular level. From the literature, it is unclear to what extent the induced hypertrophy in the CAVB dog can maintain optimal ventricular function. The majority of our dogs did not show any physical sign of decompensation in their daily existence at the slow IVR. When pressures are measured, the LV also seems to respond adequately to the increased volume: neither during steady-state rhythms of 600 ms nor during the PESP protocol was there any sign of impaired LV systolic or diastolic function. This in contrast to the pacing-induced heart failure dog, in which impaired cardiac function has been described systematically under both situations.

In the different canine models of heart failure, several reports have described huge increases in the plasma levels of neurohumoral parameters when cardiac failure develops. In time, these values remain elevated or increase further with the progression of the disease, until the situation is reversed or normalized. The return of all the neurohumoral factors to the baseline values in this model (Figure 4) indicates normalization of function: compensated hypertrophy.

Expression of ANF mRNA has been suggested to be an early indicator for ventricular decompensation, which increases progressively with development of failure. Again, the lack of expression of ANF mRNA in the ventricles of the CAVB dogs indicates absence of heart failure.

There is evidence that pressure overload–induced hypertrophy is associated with interstitial fibrosis and deterioration of function, whereas this has not been observed in volume-overload–induced hypertrophy. In canine ventricular myocardium, the volume content of collagen has been reported to be in the range of 2.5% to 4.3%. In dogs with heart failure, an increased collagen content has been described, which is accompanied by interstitial edema, disruption of collagen fibers, reduced C/F ratios, and malalignment of muscle fibers. Sabbah et al showed reactive interstitial fibrosis associated with a reduced C/F ratio. In other regions in which no fibrosis was present, normal C/F ratios of around 1.05 ± 0.03 were found. In this study, we found biventricular hypertrophy but no evidence of fibrosis or of a markedly decreased C/F ratio, suggesting a parallel increase in muscle, vessels, and connective tissue components. Information concerning RV function was not obtained in this or other studies. However, on the basis of the above observations, we believe that this chamber is also well adapted to the increased demand.

On the basis of (1) the transient increase in the neurohumoral response, which was normalized again at the moment we performed the functional tests for a second time; (2) the inability to detect expression of ANF mRNA in the ventricles; (3) the functional tests and the physical well-being of these dogs; and (4) the absence of interstitial fibrosis and the presence of a normal C/F ratio, we conclude that the majority of these dogs have compensated biventricular hypertrophy, which has been referred to as physiological or adaptive hypertrophy. During these experiments, there was 1 exception. In this dog, not included in the group comparisons, signs of heart failure were clearly seen, aldosterone levels were elevated to >2000 pmol/L, Ang II reached 660 pg/L, fibrosis was present, and ANF mRNA expression was seen in the RV (Figure 7).

Electrical Remodeling and Increased Susceptibility for TdP

In contrast to the above-described functional, structural, and neurohumoral changes, the electrophysiological adaptation has been less well documented in the different models, especially in relation to the arrhythmogenic consequences of this remodeling process. The only systematic finding is the prolongation of APD, both in vivo (MAP recordings or QT duration) and in vitro. To provide a link between the longer action potentials and the arrhythmogenic consequence of this remodeling, we tested the hypothesis that electrical remodeling predisposes the heart for EADs, ∆APD, and TdP arrhythmias in the CAVB dog.

Mild hypertrophy caused by pressure overload has been shown to prolong LV APD by 14% both in situ and in vitro. The present study shows that CAVB leads to an increase in LV APD of 32%. This different increase in APD, despite a similar amount of LV hypertrophy, could be due to differences in CL between the 2 groups: 800 ms in the pressure-overload dogs versus 1600 ms in our dogs. APD is frequency-dependent, and when our dogs are paced at comparable rates, the prolongation is less pronounced. Surprisingly, the increase in RV APD is less severe (+21%) than that of the LV APD, despite a similar absolute increase in HW and an even higher percent increase of the RV mass. These observations have also been confirmed on the cellular level, as described in the companion article. This discrepancy could indicate that the changes in the electrophysiological parameters are not only related to hypertrophy and/or bradycardia. A comparison with other models is not possible because, to the best of our knowledge, no published report has described the electrophysiological effects in dogs with RV hypertrophy. An explanation for this difference could be the different hemodynamics in the RV and LV. Stretch has the capability to shorten APD, and this opposing effect may influence the amount of prolongation. Also, for the comparison of the APD at the 2 time points, this stretch-induced shortening under AAVB circumstances may be a confounding parameter. The measured APD at control (AAVB) could be shorter, as expected on the basis of the bradycardia alone. A second reason for the difference between the LV and RV APD responses could be a different rate response in ventricular cells of the RV and LV, including M cells. At longer CLs, the prolongation of the LV APD is much longer than the RV APD. Studies in dogs and humans have shown the presence of M cells.

Consequences of Interventricular Dispersion

The consequence of the relatively smaller increase in the RV APD than in the LV APD is an increase in ∆APD in the CAVB dog. ∆APD is bradycardia-dependent: the slower the heart rate, the longer the difference between the LV and RV APD. In this study, we controlled for the CL. This increased amount of ∆APD could have important proarrhythmic con-
sequences, because it is 1 of the components necessary to induce TdP in our animal model, as we recently described.\textsuperscript{4,5} After AAVB, no EADs were present, and $\Delta$APD amounted to 40 ms. Administration of $d$-sotalol led to a lengthening of the repolarization parameters and the appearance of EADs, but not to an increase in $\Delta$APD, and no TdP could be induced. At CAVB, $\Delta$APD was increased, but EADs were still not present, with 1 exception. In that dog, TdP could be induced during baseline. $d$-Sotalol increased $\Delta$APD further because of its more pronounced effect on the LV APD and led to a higher incidence of EADs and to TdP induction in 60% of the dogs. This means not only that the baseline values are higher in CAVB but also that there is a different ventricular sensitivity for $d$-sotalol. The dogs with a low $\Delta$APD after $d$-sotalol did not develop TdP.

A criticism of our proposed role of $\Delta$APD in the genesis of TdP is the location of the dispersion. For reentry to occur, the dispersion should be localized in sites that are in close proximity.\textsuperscript{44} When $\Delta$APD is localized in the septum, this condition is fulfilled. However, $\Delta$APD could also be a marker for transmural dispersion. Shimizu and Antzelevitch\textsuperscript{45} suggested that the difference between QT$_{end}$ and QT$_{peak}$ could reflect transmural dispersion. When these 2 parameters were compared in this model, it became clear that they behave similarly and perhaps bear similar information.

LV hypertrophy and the concomitant increase in the dispersion of repolarization has also been associated with a higher vulnerability to other ventricular arrhythmias.\textsuperscript{13,17,45} This increased propensity of the hypertrophied heart to arrhythmias is often accompanied by pathological conditions, such as fibrosis, ischemia, regional differences in the degree and nature of hypertrophy, and the functional status of the heart (heart failure). Often the pathological condition(s) seem to be restricted to the LV, but little information concerning the RV is available. Therefore, it is important to emphasize that in the CAVB dog, the increased susceptibility for TdP seems to occur in hypertrophied myocardium in the absence of additional pathological conditions, in which both ventricles have been subjected to similar increases in volume. However, part of the electrical remodeling can also be caused by long-lasting bradycardia. These 2 parameters have to be separated in the near future to assess their contributions.

Regression of hypertrophy concomitant with a decrease of the electrophysiological parameters results in a markedly reduced incidence of arrhythmias to baseline.\textsuperscript{45} It would also be interesting to see whether the changes in electrophysiological parameters in our dogs can be influenced by the prevention or regression of hypertrophy. In this context, it is important to mention the study by Kreher et al\textsuperscript{46} suggesting that the adaptations leading to hypertrophy and electrophysiological changes in the aging rat heart could be 2 different processes that can operate independently.

Occurrence of TdP in dogs in the AAVB is not impossible. Studies have shown the occurrence of spontaneous TdP and polymorphic ventricular arrhythmias after administration of class III agents.\textsuperscript{47,48} However, addition of an $\alpha$-agonist\textsuperscript{47} or a several times higher dose of $d$-sotalol\textsuperscript{48} was required. This could imply that the processes that take place in the weeks after AV block are more a facilitating process than an absolute requirement for the induction of TdP. In this regard, it is of interest that in the human heart with AV block, TdP is rarely seen during acute ischemia in the setting of myocardial infarction but much more often in chronic fibrotic AV block in adults\textsuperscript{49} and children.\textsuperscript{50} It is likely that in the latter patient groups, ventricular hypertrophy is present.

**Sensitivity to $d$-Sotalol**

Prolonged repolarization facilitates the development of EADs.\textsuperscript{17} The ionic mechanisms of these EADs and of the increase in APD are not completely understood. Several reports show decreases in outward $K^+$ currents.\textsuperscript{12,16} $d$-Sotalol, like many other class III agents, blocks $I_{Kr}$ Blockade of an already diminished current may explain the higher sensitivity to $d$-sotalol of the LV hypertrophied muscle in CAVB dogs.

**Limitations of the Study**

In this study, we did not assess the time-dependent behavior of the structural and electrophysiological changes. Because we measured only at 2 time points, it is possible that the adaptations were not fully completed. Earlier studies comparing CAVB dogs at different time intervals, however, showed that there is no relation to duration of AVB and total HW.\textsuperscript{38,39} We had similar findings comparing the obtained HW at 20 weeks with 9 weeks of CAVB. In relation to the electrical effects, the response to $d$-sotalol does not change over time when we compare the experiments that were started after 2 weeks of CAVB.\textsuperscript{4-5} This suggests that the alteration responsible for the facilitated TdP induction is already present at 2 weeks. The time period between 0 and 2 weeks will be the subject of further investigations. A second limitation could be the (reproducible) quality of the signals and the random placement of the endocardial MAPs during these experiments. Especially at AAVB, it is technically difficult to assess good MAP signals. Perhaps this is due to the stretch on the ventricular wall. Random placement of the MAP catheters ignores possible intraventricular differences in APD. However, we have shown that the intraventricular difference at baseline and after $d$-sotalol is always much smaller (only 20%) than $\Delta$APD.\textsuperscript{4}

**Possible Clinical Implications**

Extrapolation from data derived from animals to the human setting should always be done with caution. Many different arrhythmia mechanisms may be generated in the hypertrophied myocardium, resulting in symptomatic arrhythmias and death.\textsuperscript{16,17} We can hypothesize that patients with ventricular hypertrophy are more prone to the proarhythmic effects (TdP) during treatment with repolarization-prolonging agents than patients without hypertrophy. A first step to be better informed about that possibility could be to stratify patients not only to QT interval, QT dispersion, and heart rate but also to localization, degree, and/or cause of ventricular hypertrophy.

**General Conclusions**

The electrical remodeling (nonuniform ventricular prolongation of APD and different sensitivity for $d$-sotalol) occurring
after chronic AVB predisposes the heart to acquired TdP, whereas the structural changes (physiological hypertrophy) are successfully aimed at maintaining cardiac function.

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