Cellular Basis of Biventricular Hypertrophy and Arrhythmogenesis in Dogs With Chronic Complete Atrioventricular Block and Acquired Torsade de Pointes

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Background—In the dog with chronic complete atrioventricular block (AVB), torsade de pointes arrhythmias (TdP) can be induced reproducibly by class III antiarrhythmic agents. In vivo studies reveal important electrophysiological alterations of the heart at 5 weeks of AVB, resulting in increased proarrhythmia. Autopsy studies indicate the presence of biventricular hypertrophy. In this study, the cellular basis of proarrhythmia and hypertrophy in chronic AVB was investigated.

Methods and Results—From chronic-AVB dogs with increased heart weights and TdP, left midmyocardial and right ventricular myocytes were isolated by enzymatic dispersion. These myocytes were significantly larger than sinus rhythm (SR) controls. In chronic AVB, the action potential spike-and-dome configuration was preserved. However, the action potential duration (APD) at 95% and 50% of repolarization of the left midmyocardium was significantly larger in chronic AVB than in SR, with little change in the right ventricle, causing enhanced interventricular dispersion of repolarization at slow pacing rates. Treatment with the class III agent almokalant increased the APD to a much larger extent in chronic-AVB than in SR myocytes and resulted in a higher incidence of early afterdepolarizations (EADs). EADs had their takeoff potential between $-35$ and $0$ mV. There was no evidence that spontaneous sarcoplasmic reticulum Ca$^{2+}$ release underlies these EADs.

Conclusions—In the dog, chronic AVB leads to hypertrophy of both right and left ventricular myocytes. The repolarization abnormalities predisposing for class III–dependent TdP in vivo are the results of cellular electrophysiological remodeling. (Circulation. 1998;98:1136-1147.)

Key Words: ventricles ■ arrhythmia ■ hypertrophy ■ action potentials ■ myocytes

The dog with chronic complete atrioventricular block (AVB) has been described as an animal model of acquired torsade de pointes arrhythmias (TdP). Clinically relevant doses of class III antiarrhythmic agents, either alone (spontaneous TdP) or in combination with programmed electrical stimulation, can evoke such a proarrhythmic response in a reproducible manner in the majority of anesthetized dogs with chronic AVB. In vivo studies show that the duration of AVB is an important determinant of the susceptibility to acquired TdP, because the latter are rarely inducible at 0 weeks of AVB (acute AVB) or at sinus rhythm (SR) but are readily inducible at $\geq 5$ weeks (chronic AVB) in most animals. The increased susceptibility to arrhythmias in chronic AVB has been related to an inhomogeneous prolongation of the monophasic ventricular action potential (in the left ventricle more than the right ventricle), leading to enhanced regional dispersion of repolarization. Furthermore, class III–dependent early afterdepolarizations (EADs) are prominent, which may explain the more frequent observation of ventricular ectopic beats in chronic-AVB dogs.

Structural adaptations accompany the altered hemodynamic load in the heart with AVB. Autopsy studies have demonstrated increased heart weight-to-body weight ratios, with significant contributions of both the right and left ventricular mass. Morphologically, the biventricular hypertrophy is characterized by an eccentric expansion with increased right and left ventricular diameters, as seen during volume overload.

Therefore, proarrhythmia in dogs with chronic AVB seems to be based on electrophysiological remodeling in the presence of bradycardia and cardiac hypertrophy. This study was designed to investigate the cellular basis of cardiac electrophysiological and structural changes in this animal model and to assess their contribution to the facilitation of TdP induction.
Methods

The animal experiments were conducted in accordance with the guidelines of the American Physiological Society and under the regulations of the Committee for Experiments on Animals of Maastricht University, Netherlands.

In Vivo Studies

Adult mongrel dogs of either sex and weighing between 22 and 32 kg were used for the experiments. AVB was created according to the procedure described by Steiner and Kovalik1 (n_dog = 9). For a complete description of the perioperative care, we refer to earlier publications.2-4

To document the induction of TdP, the class III agent almokalant was administered intravenously at a concentration of 0.12 mg · kg⁻¹ · h⁻¹. A 10-minute infusion time was used in 7 of the 9 animals with chronic AVB.5 Almokalant is a known inhibitor of the rapidly activating component (I_{Kr}) of the delayed-rectifier K⁺ current (I_{K}) and increases regional dispersion of repolarization.6,7 Surface ECG leads and right and left ventricular monophasic action potential catheter positions were obtained in a simultaneous on-line recording of the signals. TdP was defined as a polymorphic ventricular tachycardia consisting of ≥5 beats starting around the baseline in a set of prolonged QT(U) duration. These experiments were performed during anesthesia at the latest 1 week before the dogs were euthanized for cell isolation. In subsequent cellular experiments, almokalant was also used. Thus, the response to class III treatment was tested both in vivo and in vitro in dogs with documented episodes of TdP and increased heart weights.

Cell Isolation Procedure

Anesthetized chronic-AV-block (n_dog = 9) and SR dogs (n_dog = 9; similar body weights) received 10 000 IU heparin IV on thoracotomy. In the former group, AVB had been present for a duration of 6-1.5 weeks (range, 5 to 9 weeks). The hearts were excised and washed in a cardioplegic solution at 5°C to 10°C. Single right and left ventricular myocytes were isolated simultaneously according to a procedure adapted from Powell.8 Cannulas were quickly inserted into and sutured to the left anterior descending and right coronary arteries under continuous perfusion with cardioplegic solution. Subsequent perfusion was done at 37°C with (1) nominally Ca²⁺-free standard buffer solution for 10 minutes, (2) Ca²⁺-free solution with collagenase plus trypsin for 10 minutes, and (3) Ca²⁺-free solution with collagenase plus protease for 20 to 30 minutes. The epicardial surface temperature and the pH of the cardiac effluent were regularly measured to check for an adequate perfusion of the tissues. Enzymes were washed out with 0.2 mmol/L [Ca²⁺]₀ standard buffer solution for 10 minutes. Finally, the tissue was minced and the cell suspension filtered and washed. Left ventricular midmyocardial myocytes were isolated by careful harvesting of the middle third of the transmural wall of the perfused wedge, as described earlier.9 Right ventricular myocytes were harvested as a mixture from the transmural wall. Isolated myocytes were stored at room temperature in standard buffer solution.

Experimental Setup

A sample of the cell suspension was transferred to a perfusion chamber (0.5 mL) and superfused with standard buffer solution at a rate of 3 mL/min and at 37±0.5°C. Myocytes used for the experiments were selected on the basis of the following criteria: having sharp striations, clear contours, transparent cytoplasms without granulations or blebs, and a resting membrane potential below ~75 mV.

Myocytes were imaged by a video camera connected to the inverted microscope (Diaphot 300, Nikon, Inc) of the setup. The length and width of each cell were measured on a defined area of the monitor with a ×40 microscopic objective. Although the width was not constant along the length of many cells, we measured this dimension as the estimated average of the broadest and thinnest part of a cell near its middle. More than 600 left midmyocardial and >400 right ventricular cells per group were measured.

For the experiments with microelectrodes, sharp standard glass electrodes filled with 3.0 mol/L KCl (resistance, 30 to 60 MΩ) were used. Action potentials were recorded with a microelectrode amplifier (Axoclamp-2B, Axon Instruments, Inc) at cycle lengths (CLs) of 300, 400, 500, 1000, 2000, 3000, and 4000 ms. The values of action potential parameters at baseline and during treatment with almokalant presented throughout the text are the averaged measurements of 5 beats during steady-state pacing. Myocyte contractions were recorded with a video edge-motion detector (Crescent Electronics) at 16-ms temporal resolution.10 The analog output signals were digitized and stored on computer hard disk. Cell shortening was expressed in percent: amplitude in micrometers relative to cell length.

For the measurements of intracellular Ca²⁺ transients and action potentials, the whole-cell variant of the patch-clamp technique was used.11 Pipettes were pulled from borosilicate glass and had resistances of 1.0 to 3.0 MΩ when filled with 140 mmol/L KCl. Membrane potentials were recorded in the current-clamp mode (Axopatch-1D, Axon Instruments, Inc). The data were filtered at 5 kHz, read into a personal computer through an analog-to-digital converter (2 kHz), and stored for later analysis. The data acquisition program also controlled the command potential and various components of the intracellular Ca²⁺ ([(Ca²⁺])₀) measurement system (Fastlab Software, Indec Systems). The pipette solution contained Fluo-3 and Fura red (Molecular Probes).12 The combined use of these fluorescent indicators was validated in confocal microscopy.13 Our experimental setup for [Ca²⁺]₀ measurements has been described elsewhere.14

Solutions

The standard buffer solution contained (in mmol/L) NaCl 145, KCl 4.0, CaCl² 1.8, MgCl² 1.0, NaHPO₄ 1.0, glucose 11.1, and HEPES 10, pH 7.4 with NaOH at 37°C and bubbled with O₂. In the cold cardioplegic solution, KCl was set to 8.0 mmol/L. For cell isolation, collagenase (1.1 mg/mL; type A, Boehringer Mannheim) and protease (0.05 mg/mL; type XIV, Sigma Chemical Co) were used in the presence of BSA (1.0 mg/mL). The patch-pipette solution contained (in mmol/L) potassium aspartate 120, KCl 20, MgCl² 0.5, MgATP 5, NaCl 10, HEPES 10, Fluo-3 0.03, and Fura red 0.07, pH 7.2 with KOH. All of the chemicals were reagent grade and cell-culture tested.

Statistical Analysis

The data are expressed as mean±SD. Intergroup comparisons were made by Student’s t test for unpaired (chronic AVB versus SR; right versus left ventricle) and paired (baseline versus treatment) data groups, respectively, after testing for the normality of distribution. The χ² test was used when the data were presented as a proportion. Differences were considered significant if P<0.05.

Results

In Vivo Studies

In our companion study,4 we report on the electrophysiological consequences of clinically relevant doses of d-sotalol in dogs with AVB. In preliminary cellular experiments of the present study, we chose to use almokalant because this agent exhibits a high specificity of I_{Kr} inhibition at nanomolar to micromolar concentrations,9 whereas d-sotalol may also exert other actions.7,15 Accordingly, the proarrhythmic potential of almokalant was tested in vivo (n_dog = 7). An example of the electrophysiological consequences of almokalant treatment and subsequent spontaneous TdP induction is shown in Figure 1. At 4±2 weeks of AVB, the class III agent increased the QT interval from 415±90 to 545±105 ms (P<0.05) without significant change of the CL of the idioventricular rhythm (from 1545±300 to 1655±295 ms; P=NS). Almokalant caused an increase of the monophasic action potential...
Cellular Basis of Biventricular Hypertrophy

When weighed directly after excision, the hearts of chronic-AVB dogs \( (n_{\text{dogs}}=9; \pm 1 \text{ weeks of AVB}) \) were significantly heavier than those of SR controls \( (n_{\text{dogs}}=9); 285\pm25 \text{ vs } 222\pm59 \text{ g, respectively (}P<0.05\text{). When corrected for body weight, the difference in heart weight remained significant: } 10.3\pm1.3 \text{ vs } 8.5\pm1.5 \text{ g/kg, respectively (}P<0.05\text{). Myocytes were successfully isolated from the right ventricular free wall and the left ventricular midmyocardium of all dogs. Representative photomicrographs of single left midmyocardial cells are shown in Figure 2. Right ventricular myocytes showed a similar morphology both in chronic AVB and in SR. All cells were quiescent during superfusion with the standard buffer solution containing 1.8 mmol/L [Ca\(^{2+}\)]. In the SR group, right ventricular myocytes were of equal length and width compared with left midmyocardial myocytes: cell length was 140\pm10 and 140\pm16 \text{ \mu m (}P=\text{NS)}, and cell width was 24\pm2 and 25\pm2 \text{ \mu m, respectively (}P=\text{NS}). In the chronic-AVB group, myocytes from both ventricles were significantly longer than SR controls, being 172\pm8 \text{ \mu m (right ventricle)} and 158\pm7 \text{ \mu m (left midmyocardium; both } P<0.05\text{, chronic AVB versus SR). The difference in cell length of right versus left ventricle in chronic AVB was statistically significant (}P<0.05\text{). By contrast, the width of these cells was not different from SR controls: 26\pm1 \text{ and } 26\pm2 \text{ \mu m, right versus left ventricle, respectively (}P=\text{NS). Frequency distributions for cell length and width are shown in Figure 3.}

Action Potentials in Single Myocytes From Dogs With Chronic AVB

Action potentials were recorded at various physiologically relevant pacing CLs with the microelectrode technique in a...
total of 51 myocytes (chronic AVB: \( n_{\text{dog}} = 7, n_{\text{cell,LV}} = 13, n_{\text{cell,RV}} = 9 \); SR: \( n_{\text{dog}} = 8, n_{\text{cell,LV}} = 15, n_{\text{cell,RV}} = 14 \)). Representative action potentials of the 4 different cell groups are shown in Figure 4. In SR, the spike-and-dome configuration was more pronounced in right ventricular than left midmyocardial action potentials and more pronounced at longer CLs, as expected. In chronic AVB, this pattern was preserved or slightly accentuated (Figure 4).

In SR, the APD at 95% of repolarization (APD95) increased on slowing of the pacing rate, as expected. Pooled data are shown in Figure 5. Chronic AVB significantly steepened this APD95/CL relationship in the left midmyocardial myocytes.
and SR groups. In total, 452 and 403 right and 607 and 676 left ventricular cells are measured in chronic AVB and SR, respectively. Relatively higher numbers of long cells (>150 μm) can be observed in chronic AVB.

\( P<0.05 \), chronic AVB versus SR) but not in the right ventricular cells \( P=\text{NS}, \) chronic AVB versus SR. The interventricular difference of \( \text{APD}_{95} \) was therefore significant at all CLs (Figure 5). The same was found for the APD at 50% of repolarization \( \text{APD}_{50} \). This parameter increased to the same extent as \( \text{APD}_{95} \) in chronic AVB, again with significant changes in the left midmyocardial but not the right ventricular cells. The maximal velocity of repolarization during phase 3 (measured as the most negative first derivative of the membrane potential in that phase) was not different between chronic AVB and SR in both ventricles (data not shown).

**Figure 3.** Frequency distributions for lengths and widths of right ventricular free wall and left midmyocardial myocytes in chronic-AVB and SR groups. In total, 452 and 403 right and 607 and 676 left ventricular cells are measured in chronic AVB and SR, respectively. Relatively higher numbers of long cells (>150 μm) can be observed in chronic AVB.

**Figure 4.** Action potentials of single myocytes from dogs with chronic AVB as a function of pacing CL. In each panel, pacing CLs are 500, 2000, and 4000 ms from left to right. Action potentials of left midmyocardial myocytes are more prolonged in chronic AVB than SR. APD is larger in left than right ventricular cells in both conditions, especially at long CL. Micro-electrode technique.
In an additional population of 32 myocytes of the same dogs, we recorded action potentials during whole-cell patch clamp (chronic AVB: n_{celL, LV} = 7, n_{celL, RV} = 8; SR: n_{celL, LV} = 10, n_{celL, RV} = 5). We confirmed the differences between APD of left versus right ventricle and chronic AVB versus SR under otherwise similar conditions (data not shown).

We also examined the peak contraction amplitudes and $[\text{Ca}^{2+}]_i$ accompanying the action potentials. At the pacing CL of 1000 ms, the peak contraction amplitude measured 4.3 ± 1.9% versus 3.1 ± 3.7% in right ventricular cells ($P = \text{NS}$) and 7.2 ± 2.2% versus 5.1 ± 2.1% in left midmyocardial myocytes ($P < 0.05$, chronic AVB versus SR, respectively). Contraction durations were not different in these cell groups. $[\text{Ca}^{2+}]_i$ peaked at 460 ± 171 versus 517 ± 102 nmol/L in the right ventricular mixture ($P = \text{NS}$, chronic AVB versus SR, respectively) and at 457 ± 146 versus 496 ± 107 nmol/L in the left midmyocardium ($P = \text{NS}$).

Increased Sensitivity of Chronic-AVB Myocytes to Almokalant

We treated both SR and chronic-AVB myocytes with 1 μmol/L almokalant. A significant prolongation of the action potential was observed in all cells tested (n = 29; microelectrode technique), which was most pronounced at long pacing CLs. Representative examples of action potentials of chronic-AVB myocytes during treatment with almokalant are shown in Figure 6. In chronic-AVB cells, the relative increase of the APD$_{95}$ during almokalant (in the absence of EADs) was much larger than in SR, independent of the chamber. This is illustrated by the pooled data of APD$_{95}$ shown in Figure 7A. In all cells, the APD$_{50}$ increased to the same extent as the APD$_{95}$.

In the myocytes studied with patch electrodes (n = 32), we found comparable responses to almokalant; likewise, the average increase of the APD was much larger in the chronic-AVB than the SR cells (Figure 7B).

Action potential prolongation was often followed by the occurrence of EADs in chronic-AVB myocytes but not in SR cells, with the microelectrode as well as the patch-electrode technique. The Table expresses this increased sensitivity as a larger proportion of cells in which EADs were observed during treatment with almokalant. EADs started at significantly more negative levels in the right ventricle (n_{EAD} = 50) than the left midmyocardium (n_{EAD} = 100): -30 ± 2 versus -19 ± 5 mV, respectively ($P < 0.05$), whereas the action potentials analyzed for this purpose had equal resting membrane potentials. EAD amplitudes were larger in right versus left ventricular myocytes: 21 ± 6 versus 10 ± 5 mV ($P < 0.05$).

We addressed the question of whether spontaneous Ca$^{2+}$ release from the sarcoplasmic reticulum could underlie depolarization (sometimes lasting several seconds) that were followed by ≥1 EADs before a rapid repolarization. Large beat-to-beat variability of the APD often characterized the class III action, as is shown in Figure 8.

Characteristics of the Almokalant-Induced EADs in Chronic AVB

The takeoff potentials of almokalant-induced EADs in chronic AVB ranged between −35 and 0 mV. On average, EADs started at significantly more negative levels in the right ventricle (n_{EAD} = 50) than the left midmyocardium (n_{EAD} = 100): −30 ± 2 versus −19 ± 5 mV, respectively ($P < 0.05$), whereas the action potentials analyzed for this purpose had equal resting membrane potentials. EAD amplitudes were larger in right versus left ventricular myocytes: 21 ± 6 versus 10 ± 5 mV ($P < 0.05$).

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these EADs, as described earlier for isoproterenol-induced EADs. Figure 9 illustrates typical findings for 2 left midmyocardial myocytes. \([\text{Ca}^{2+}]_i\) rose rapidly on depolarization, reflecting \([\text{Ca}^{2+}]_i\) release from the sarcoplasmic reticulum. This release was followed by a rapid but incomplete decline of \([\text{Ca}^{2+}]_i\), and \([\text{Ca}^{2+}]_i\) typically remained elevated at \(\approx 30\%\) above baseline values as long as the membrane potential did not recover completely (Figure 7).
9A). EADs during this phase, however, were not associated with distinct new [Ca\textsuperscript{2+}] \textsuperscript{i} transients. Small fluctuations of [Ca\textsuperscript{2+}] \textsuperscript{i} were sometimes observed, in amplitude always <5% of the initial [Ca\textsuperscript{2+}] \textsuperscript{i} transient. In the case of cell shortening, the normal twitch contraction was followed by a relaxation phase during which cell length attained near-resting levels. However, as with the [Ca\textsuperscript{2+}] \textsuperscript{i}, full relaxation awaited full repolarization of the action potential (Figure 9B). In a few cells and only when large-amplitude EADs were generated, we could discern small early aftercontractions that followed the EAD upstroke with a delay of several tens of milliseconds (Figure 9B, arrow). Similar results on [Ca\textsuperscript{2+}] \textsuperscript{i} and cell shortening during EADs were obtained in 30 cells treated with almokalant.

### Discussion

**Cellular Hypertrophy in Chronic AVB**

The present study confirms the earlier finding of increased heart weight-to-body weight ratios in adult dogs with chronic AVB.\textsuperscript{4} The hearts are enlarged consistent with eccentric hypertrophy, as described for volume overload.\textsuperscript{20} The hypertrophy is related to increased lengths of both right and left ventricular myocytes. Myocyte growth has been observed in most large-animal models of cardiac hypertrophy reported in the literature (eg, References 21 through 23). We found that the biventricular hypertrophy of chronic AVB was not a homogeneous process: growth responses were larger in the right than left ventricular myocytes (+23% versus +13% for cell length, respectively), supporting the autopsy finding of a larger relative increase of the right than the left ventricular weight.\textsuperscript{4} This differential ventricular growth response resembles the findings of another animal model of biventricular hypertrophy related to volume overload (aorto-caval fistula).\textsuperscript{22} Also, in pigs with heart failure due to rapid pacing, myocytes were larger in the right than left ventricle.\textsuperscript{24} The preponderance of right ventricular cell growth in chronic AVB may reflect a greater impact of the altered hemodynamics on that chamber after the transition from SR to idioventricular rhythm.

As to the functional characteristics of chronic-AVB myocytes, we demonstrated that peak amplitudes of contraction and [Ca\textsuperscript{2+}] \textsuperscript{i}, at 1000-ms pacing CL were not different from SR cells (or, if anything, were slightly increased). These data, which are the subject of further investigations, provide additional support for the notion, based on in vivo hemodynamic measurements,\textsuperscript{7} of a compensated form of cardiac hypertrophy.

**Cellular Electrophysiological Remodeling in Chronic AVB**

Our data show that myocytes from dogs with chronic AVB have (1) an intrinsic disturbance of repolarization with prolonged action potentials at baseline, (2) interventricular differences of repolarization, and (3) an increased susceptibility to class III antiarrhythmic agents.

**Intrinsic Disturbance of Repolarization**

Prolongation of the left ventricular action potential is a consistent observation in myocardial hypertrophy of different causes in several species.\textsuperscript{25–27} Cellular electrophysiological data on right ventricular hypertrophy are scarce. In pressure-overload–induced right ventricular hypertrophy, increases of the APD have been reported.\textsuperscript{28} Many of the sarcolemmal ion channels, exchangers, and pumps as well as intracellular ion transporters that shape the action potential under normal conditions can show functional defects leading to delayed repolarization in hypertrophy.\textsuperscript{29,30} Other investigators have shown that this action potential prolongation predisposes to an increased susceptibility to EADs\textsuperscript{31} and ventricular tachyarrhythmias.\textsuperscript{32,33} As yet, the ionic basis of action potential changes in chronic AVB remains to be elucidated. The APD\textsubscript{95} and APD\textsubscript{50} increased to a similar extent, indicating that the delay in repolarization was most likely due to a disturbance at the plateau level. From the data in this study, a prominent role for the transient outward current (I\textsubscript{to}) appears unlikely: the plateau arrest can be observed that lasts for full CL and is overcame only after pacing stimulus of next action potential. In consecutive beats, APD\textsubscript{95} varies from 500 to 900 ms. This is an example of temporal dispersion of repolarization within a single cell.
of the action potential and an increase of the APD, which has been linked to the generation of lethal ventricular arrhythmias. Additional insights into the ionic mechanisms of action potential prolongation in chronic AVB come from the experiments with almokalant. Carmeliet demonstrated that in rabbit ventricular myocytes, $I_{Kr}$ is fully blocked by 1 μmol/L of the agent, and we have confirmed this in normal dog ventricular myocytes (data not shown). After block of $I_{Kr}$ during almokalant treatment, we found that the APD was much larger in chronic-AVB left and right ventricular myocytes than in SR controls (Figure 7). This implies that ionic currents other than $I_{Kr}$ contribute to the abnormal repolarization of chronic AVB, even though we cannot exclude that $I_{Kr}$ also plays a role.

**Interventricular Differences of Repolarization**

To the best of our knowledge, this is the first study to compare action potential characteristics of single hypertrophied myocytes isolated from both the right and left ventricles of the same dog. Interventricular differences of the action potential have been known to exist in the normal myocardium of dogs with SR, and this was confirmed by the present study. In chronic AVB, we found larger APDs in left midmyocardial than right ventricular myocytes at baseline, in contrast to the larger degree of hypertrophy in the latter than the former cells. Yet, the administration of almokalant increased the APD to a similarly high level in both ventricles. Thus, the presence of intrinsic repolarization disturbances was unmasked in right ventricular myocytes. At least 3 explanations could account for the interventricular differences of repolarization at baseline: (1) there was a different electrophysiological remodeling in both ventricles, (2) the charge carriers involved were the same but their functional alterations mounted to different amplitudes, or (3) a finer balance of currents existed during the plateau phase because of a decrease in outward current and/or an increase in inward current.

**Increased Susceptibility to Almokalant**

Chronic-AVB cells showed an increased susceptibility to action potential prolongation and EAD generation during class III treatment. These EADs were observed at long pacing CLs and had takeoff membrane potentials ranging between −35 and 0 mV. Very small changes in the balance of inward and outward currents during the high-resistance plateau can determine the appearance or absence of EADs. At least 2 possibilities could explain the increased number of EADs found in this study: (1) they occurred because the APD was initially prolonged and/or (2) the charge carrier(s) responsible for their generation were more prominent in myocardial hypertrophy, eg, increased L-type Ca$^{2+}$ current ($I_{CaL}$) or more spontaneous sarcoplasmic reticulum Ca$^{2+}$ release. If the former is true, it follows that any intervention causing the action potential to shorten would lead to the diminished appearance of EADs. Preliminary results with the agent levromakalim, an $I_{K-ATP}$ activator, support this hypothesis: we found that in consecutive action potentials with EADs, levromakalim initially

![Cellular handling of [Ca$^{2+}$]$_i$ during class III–dependent EADs in chronic AVB. A, Simultaneous recording of action potential and [Ca$^{2+}$]$_i$ with current clamping in whole-cell variant of patch-clamp technique and with fluorescent indicators Fluo-3 and Fura red in internal pipette solution. B, Action potential (microelectrode technique) is recorded simultaneously with cell shortening. A and B, Left ventricular midmyocardial cells were treated with almokalant at 1 μmol/L during stimulation at a pacing CL of 4000 ms, leading to generation of EADs. Full relaxation of [Ca$^{2+}$] and contraction, in A and B, respectively, was delayed until full repolarization of action potential. Distinct or high-amplitude peaks of [Ca$^{2+}$]$_i$ (ie, Ca$^{2+}$ aftertransients) were not observed during EADs, as illustrated in A. Small aftercontractions following upstroke of EADs were rarely observed. Example is shown in B (arrow).](http://circ.ahajournals.org/)}
caused a decrease of the APD, followed by the disappearance of the EADs and a further shortening of the action potential. The finding that DADs also remained absent during this action potential shortening was an argument against the possibility of spontaneous sarcoplasmic reticulum Ca\(^{2+}\) release to underlie the class III–dependent EADs of chronic AVB. Indeed, the characteristics of cytoplasmic Ca\(^{2+}\) during the EADs confirmed this notion. We did find that the relaxation of [Ca\(^{2+}\)], and cell shortening was slow during action potentials with EADs. The decline of [Ca\(^{2+}\)], always paralleled the repolarization regardless of the APD, which suggests that the voltage dependence of cellular Ca\(^{2+}\) extrusion was maintained as under normal conditions. Our results in chronic-AVB myocytes resemble those of cesium chloride–induced EADs in normal ferret ventricular muscle\(^{37}\) and class III–induced EADs in normal rabbit ventricular myocytes.\(^{38}\)

Although our data support an important role for the initial action potential prolongation, we cannot exclude the possibility that intrinsic changes of \(I_{\text{Ca,L}}\) may be involved in the increased number of EADs.

Our finding that EAD amplitudes were larger in right than in left ventricular myocytes could be related to the lower takeoff potentials in the former cells. If indeed \(I_{\text{Ca,L}}\) is the major charge carrier of these EADs, its triggering at lower takeoff potentials would most likely lead to larger current amplitudes.

### Cellular Basis of In Vivo Proarrhythmia

Our data show that the interventricular differences of APD were most pronounced at the longer pacing CLs of \(\geq\)2000 ms, measuring maximally 100 ms at baseline. Because in most cases the CLs of the idioventricular rhythms of the dogs were \(>1500\) ms, we consider the cellular findings relevant for the in vivo situation and find them consistent, at least qualitatively, with the monophasic action potential findings in the same dogs before they were euthanized. We do not believe that interventricular dispersion itself is the direct cause for the initiation of torsade de pointes, but because it is always present during arrhythmogenesis, it probably reflects the existence of dispersion of more closely juxtaposed regions. When using almokalan in our experiments, we found that this agent promoted temporal heterogeneity of repolarization with varying APDs and EADs that appeared in an on-and-off fashion in consecutive beats during steady-state pacing. Dispersion could be enhanced even more if the heterogeneity of cellular repolarization occurred out of phase in various regions of the myocardium. We have demonstrated such spatial dispersion in vivo.\(^3\)

Another important factor involved in the occurrence of TdP is ventricular ectopy. In vivo recordings in the dog with chronic AVB confirm the importance of ventricular ectopic beats (Figure 1)\(^2,4\); however, until now their origin and mechanisms remain obscure. Although others have demonstrated triggered activity on EADs in Purkinje fibers during in vitro conditions that mimic proarrhythmia in vivo,\(^{39–41}\) it can be concluded from the present study that in single cells from the working myocardium, abnormal impulse generation is limited to EADs that do not trigger new action potentials in these same cells. This conclusion is based on our findings that the EADs always (1) arose from a takeoff membrane potential less negative than \(-35\) mV (above the threshold for full activation of Na\(^{+}\) current) and did not show a rapid upstroke as in normal action potentials; (2) had an amplitude of \(<30\) mV; (3) prolonged the repolarization but did not take the shape of normal action potentials; (4) were incapable of inducing normal, if any, contractions; and (5) behaved differently from the EADs observed in Purkinje fibers or in ventricular myocytes with takeoff levels closer to the resting membrane potential. Thus, the characteristics of these membrane responses do not match our definition of the ventricular action potential.

DAD-triggered action potentials or abnormal automaticity were also not observed in the chronic-AVB cells. This all may mean that the substrate for extrasystolic activity involved in the initiation of TdP is multicellular (eg, phase 2 reentry) or includes the Purkinje network. In addition, the experimental setup chosen for this study lacks many of the components affecting arrhythmogenesis in vivo, such as adrenergic agonists, stretch, and sudden rate changes. For this reason, the absence of spontaneous action potentials in the single myocyte does not exclude their generation in vivo.

### Limitations of the Study

One of the aspects of this study was to compare the action potential characteristics of myocytes from the left mid-myocardium with those from the right transmural ventricular wall. One could argue that this comparison is not fully adequate, given the findings made in tissue studies that the shape of the ventricular action potential and the CL dependence of its duration vary across the free wall of both chambers.\(^42\) Because of the thinness of the right ventricular wall, we found it difficult to isolate the cells according to their transmural site of origin in that chamber. Nevertheless, at long pacing CLs, right ventricular myocytes consistently had a typical action potential configuration that was different from that of the left midmyocardial cells. In addition, the range of APD measured in left and right ventricular cells, eg, at a CL of 2000 ms, showed an overlap of only 10% in chronic AVB and of 20% in SR. A “bad pick” of only epicardial or endocardial cells from the right ventricular mixture might explain this finding, but this is unlikely. A more plausible explanation is that the APD differences of midmyocardial versus epicardial and endocardial myocytes are much less in the right than the left ventricle. The comparison of right transmural versus left transmural ventricular myocytes (instead of right transmural versus left midmyocardial cells, as made now) would most likely underestimate the interventricular dispersion of repolarization present in these hearts and was therefore not applied.

When we compared the action potential characteristics of our right ventricular mixture with the data presented by Sicouri and Antzelevitch,\(^42\) we found that the majority of our cells (93% for SR and 67% for chronic AVB) had a
steep APD/CL relationship, which would identify them as right ventricular midmyocardial cells.

Conclusions
We conclude that AVB of chronic duration leads to inhomogeneous hypertrophy of right and left ventricular myocytes. Cellular hypertrophy is associated with an intrinsic defect of repolarization in the right and left ventricles. APD differences between left midmyocardial and right ventricular myocytes cause an enhanced inter-ventricular dispersion of repolarization, which is most pronounced at the longer pacing CL. Almokalant increases the APD to a much larger extent in chronic AVB than in SR, thus further unmasking the presence of intrinsic repolarization disturbances. In addition, chronic-AVB cells of both ventricles show an increased susceptibility to EAD generation. Although the present study does not elucidate the ionic basis of these action potential differences or the genesis of ventricular ectopic beats, it appears that phenotypic alterations of repolarization are involved in the ventricular arrhythmias and sudden cardiac death of dogs with chronic AVB.

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