Electropharmacological Characterization of Cardiac Repolarization in German Shepherd Dogs With an Inherited Syndrome of Sudden Death: Abnormal Response to Potassium Channel Blockers

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OBJECTIVES
This study sought to determine whether abnormal ventricular repolarization is implicated in cardiac arrhythmias of German shepherd dogs with inherited sudden death.

BACKGROUND
Moisé et al. (9) have identified German shepherd dogs that display pause-dependent lethal ventricular arrhythmias.

METHODS
Ventricular repolarization was studied both in vivo using electrocardiogram recordings on conscious dogs and in vitro with a standard microelectrode technique performed on endomyocardial biopsies and Purkinje fibers. Pharmacological manipulation was used to evaluate the role of potassium channels.

RESULTS
In control conditions, electrocardiogram parameters were similar in both groups of dogs, except for the PR interval (18% longer in affected dogs, p < 0.05). Injection of d,l-sotalol (2 mg/kg) prolonged QT interval more in affected dogs (+14%, n = 9) than it did in unaffected dogs (+6%, n = 6, p < 0.05) and increased the severity of arrhythmias in affected dogs. In vitro, in control conditions, action potential duration (APD90) of endomyocardial biopsies and Purkinje fibers were significantly longer in affected dogs (respectively 209 ± 3 ms, n = 30 and 352 ± 15 ms, n = 17) than they were in unaffected dogs (197 ± 4 ms, n = 25 and 300 ± 9 ms, n = 30) at a pacing cycle length (PCL) of 1,000 ms. This difference increased with PCL. The kinetics of adaptation of APD90 to a change in PCL was faster in affected dogs. D,l-sotalol (10⁻⁴ and 10⁻³M) increased APD90 in both groups of dogs, but this increase was greater in affected dogs, with the occurrence of triggered activity on Purkinje fibers. E-4031 (10⁻⁷ and 10⁻⁶ M), an IKr-blocker, increased APD90 similarly in both groups of dogs. Chromanol 293B (10⁻⁶ and 10⁻⁵M), an IKs-blocker, increased significantly APD90 in unaffected dogs but had no effect in affected dogs.

CONCLUSIONS
These results support the hypothesis of an abnormal cardiac repolarization in affected dogs. The effects of 293B suggest that IKs may be involved in this anomaly. (J Am Coll Cardiol 2000;36:939–47) © 2000 by the American College of Cardiology

Sudden cardiac death is a major cause of death in developed countries. Most commonly, the underlying mechanism is ventricular tachycardia or fibrillation in association with organic heart disease (1). However, cardiac arrhythmia-induced sudden death was also observed in subpopulations of patients with structurally normal hearts. These included patients with various forms of long QT syndrome (2) but also patients with delayed ventricular repolarization such as patients with short-coupled ‘torsades de pointes’ (3), catecholamine-induced ventricular tachycardia (4) or idiopathic ventricular fibrillation, so called ‘Brugada syndrome’ (5). In these patients, sudden death usually occurs at a young age and sometimes without any previous alarming symptoms. Finally, lethal arrhythmias were also suggested to contribute to some cases of sudden infant death syndrome (6). The identification of patients at risk is difficult because the mechanisms responsible for the lethal arrhythmias remain largely unknown mainly because of a lack of suitable animal models (7,8).

Recently, one of us has established a colony of German shepherd dogs with ventricular arrhythmias and sudden death (9), inherited as a simple autosomal dominant with incomplete penetrance or polygenic trait (10). In these animals, arrhythmias and death occur at a young age (10) and are not associated with a structural heart disease. The phenotype spectrum of the arrhythmias is wide, ranging from infrequent premature ventricular beats to nonsustained polymorphic ventricular tachycardia, with sometimes a ‘torsades de pointes’-like morphology. Usually (more than 80% of cases) the incidence of arrhythmias is increased at low heart rates (HR) and after sinus pauses. All these features are typically those observed in patients with long QT syndrome although prolongation of the QT interval was not found in affected dogs. However, that QT interval is normal does not mean that the affected dogs do not have abnormal ventricular repolarization. Indeed, affected dogs have more

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frequent notching of the T-waves (10) than unaffected dogs. Moreover, left ventricular Purkinje fibers isolated from affected dogs exhibit early afterdepolarizations (EADs) and triggered activity (TA) (11,12), a potential mechanism for lethal arrhythmias (7,13).

We, therefore, decided to further investigate the ventricular repolarization of these German shepherds. We evaluated the effects of d,l-sotalol, a beta-adrenergic blocking agent also known to block potassium channels (14,15), on action potentials (AP) and electrocardiogram (ECG). We finally assessed the in vitro effects of two specific blockers of the rapid and the slow components of the delayed rectifier potassium current (I\textsubscript{K}): E-4031, an I\textsubscript{Kr}-blocker (16) and chromanol 293B, and I\textsubscript{Ks}-blocker (17).

**METHODS**

This investigation conforms with the position of the American Heart Association on research animal use, adopted by the American Heart Association November 11, 1984.

**Animals.** The dogs used for this study were part of a colony implanted in the Veterinary School of Nantes, France, and developed from five affected dogs coming from the colony of Cornell University (9). The incidence and severity of cardiac arrhythmias were assessed using Holter monitoring and challenges with phenylephrine (18). The affected group (14 dogs) was comprised of inbred German Shepherds that displayed frequent ventricular premature beats and nonsustained ventricular tachycardia spontaneously or after phenylephrine injections. The unaffected group (29 dogs) was comprised of 20 inbred German shepherds, 3 Beagles, 6 mongrel dogs from two F1 generations—resulting from the crossing of affected dogs with pure Labradors—that did not display arrhythmias.

**In vivo studies.** Nine unaffected dogs (29 to 52 weeks old; 38 ± 2) and six affected dogs (30 to 49 weeks old; 38 ± 3)
Table 1. Evolution of Arrhythmias in Affected Dogs During D,l-sotalol Protocols (Maximum Number of Arrhythmias/min)

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Before Sotalol</th>
<th>After Sotalol</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAN742</td>
<td>26 VPB</td>
<td>50 VPB</td>
</tr>
<tr>
<td></td>
<td>5 doubles</td>
<td>10 doubles</td>
</tr>
<tr>
<td>M3</td>
<td>25 VPB</td>
<td>44 VPB</td>
</tr>
<tr>
<td></td>
<td>5 doubles</td>
<td>1 VT</td>
</tr>
<tr>
<td>M7</td>
<td>1 VPB</td>
<td>8 VPB</td>
</tr>
<tr>
<td>N12</td>
<td>—</td>
<td>5 VPB</td>
</tr>
<tr>
<td>N17</td>
<td>1 VPB</td>
<td>5 VPB</td>
</tr>
<tr>
<td></td>
<td>2 doubles</td>
<td>5 VPB</td>
</tr>
<tr>
<td></td>
<td>3 triplets</td>
<td>3 VT</td>
</tr>
</tbody>
</table>

= no arrhythmia; VPB = ventricular premature beat; VT = ventricular tachycardia.

were used to evaluate the ECG effects of d,l-sotalol. Three leads ECG (I, II, III) were continuously monitored on conscious dogs lying quietly in a hammock. Measurements were made on lead II. They included HR, QT interval (measured as described by Weissenburger et al. [19]) and PR duration. Baseline measurements were made at least 30 min after the end of the instrumentation. Then, atenolol (Tenormine i.v., Zeneca Pharma, France) was injected as an intravenous (IV) bolus of 2 mg/kg (similar HR) to study only the class III effects of d,l-sotalol, which is also a beta-blocker, and to decrease HR, which may facilitate the occurrence of bradycardia-dependent arrhythmias in affected dogs (12), those that were of interest for this study. D,l-sotalol (Sotalex, i.v., Bristol-Myers-Squibbs, France) was injected twice as an IV bolus of 2 mg/kg, 15 (time t₀) and 45 min after the IV bolus of atenolol. Electrocardiogram measurements were done 15, 30, 45 and 60 min after t₀.

**In vitro studies.** Endomyocardial biopsies. Dogs were anesthetized with an IV bolus of ketamine (0.7 mg/kg; Imalgène, Rhône-Mérieux, France) and xylazine (0.7 mg/kg; Rompun, Bayer, Germany). The biotome (Cordis 7F/2.2 mm; Cordis, S.A., France) was advanced to the right ventricle through the right jugular vein. Each biopsy (4 to 5 in each dog) excised from the septal subendocardium was immersed quickly in a cold Tyrode’s solution containing (in mM): NaCl, 124; NaHCO₃, 27; NaH₂PO₄, 1.8; KCl, 4; MgCl₂, 0.5; CaCl₂, 2.7; glucose, 5 (pH 7.4).

**Purkinje fibers.** Dogs were anesthetized with pentobarbital sodium (30 mg/kg IV, Pentobarbital sodique, Sanofi, France). The hearts were quickly removed through a left thoracotomy and immersed in a cold Tyrode’s solution (see preceding text). Purkinje fibers were dissected from both ventricles.

**Experimental protocols.** The tissues were mounted in a Lucite chamber perfused with an oxygenated (95% O₂–5% CO₂) Tyrode’s solution warmed to 37 ± 0.5°C. Transmembrane recordings were obtained as previously described (20) and analyzed with the software Acquisi (Biologic, France). The tissues were allowed to recover for at least 1 h before the experiments were started. During this period, they were driven at a pacing cycle length (PCL) of 1,000 ms by field or bipolar stimulation through Teflon-coated silver wire electrodes. Stimulus pulse width was 0.5 to 1.5 ms, and amplitude was twice diastolic threshold.

To study the effects of pacing on repolarization, the preparations were driven at PCL decreasing from 8,000 to 300 ms. Action potential characteristics were measured at steady state for each PCL. We measured the resting potential—or the maximal diastolic potential for Purkinje fibers—the action potential amplitude, the maximum upstroke velocity of

**Table 2. Electrophysiological Characteristics of Action Potentials Recorded From Canine Endomyocardial Biopsies and Purkinje Fibers Paced at a Cycle Length of 1,000 ms in Control Conditions**

<table>
<thead>
<tr>
<th>Endomyocardial Biopsies</th>
<th>Purkinje Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unaffected</strong>&lt;br&gt; (n = 25, 17 Dogs, 40 ± 4 Weeks)</td>
<td><strong>Affected</strong>&lt;br&gt; (n = 30, 12 Dogs, 42 ± 3 Weeks)</td>
</tr>
<tr>
<td>RP/MDP (mV)</td>
<td>−92 ± 1</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>119 ± 1</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (V/s)</td>
<td>360 ± 28</td>
</tr>
<tr>
<td>Phase 1 (mV)</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Phase 2 (mV)</td>
<td>104 ± 1</td>
</tr>
<tr>
<td>APD&lt;sub&gt;90&lt;/sub&gt; (ms)</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>APD&lt;sub&gt;50&lt;/sub&gt; (ms)</td>
<td>156 ± 3</td>
</tr>
<tr>
<td>APD&lt;sub&gt;30&lt;/sub&gt; (ms)</td>
<td>184 ± 4</td>
</tr>
<tr>
<td>APD&lt;sub&gt;0&lt;/sub&gt; (ms)</td>
<td>197 ± 4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, rounded off to nearest whole number. The number of experiments and dogs and their mean age are given in parentheses.

APA = action potential amplitude; APD<sub>90</sub>, APD<sub>50</sub>, APD<sub>30</sub> and APD<sub>0</sub> = action potential duration at 30, 50, 70 and 90% of full repolarization, respectively; MDP = maximum diastolic potential (for Purkinje fibers); phase 1 = phase 1 amplitude; phase 2 = phase 2 amplitude; RP = resting potential (for endomyocardial biopsies); V<sub>max</sub> = maximum upstroke velocity of phase 0.

*<i>p < 0.05</i> and †<i>p < 0.01</i> versus unaffected (Student t test).
stroke velocity of phase 0, the phase 1 and phase 2 amplitudes (differences between resting potential or maximal diastolic potential and the potential at the end of phase 1 or the top of phase 2, respectively) and the action potential duration (APD) at 30% (APD_{30}), 50% (APD_{50}), 70% (APD_{70}) and 90% (APD_{90}) of full repolarization. The same pacing protocol was performed after 15 min of superfusion with dl-sotalol (10^{-5} and 10^{-4}M), E-4031 (10^{-7} and 10^{-6}M, custom synthesized according to [21]) or chromanol 293B (293B; 10^{-6} and 10^{-5}M, gift from Hoechst, Germany). E-4031 and 293B were dissolved in distilled water and dimethyl-sulfoxide, respectively. Between the pacing protocols, the tissues were driven at a PCL of 1,000 ms.

To study the kinetics of adaptation of repolarization to sudden changes in pacing rate, the samples were driven at PCL increasing abruptly from 300 to 1,000 ms and from 1,000 to 8,000 ms or decreasing abruptly from 8,000 to 1,000 ms and from 1,000 to 300 ms. During the periods of adaptation after the changes in PCL (up to 4 min), all the AP were recorded. Their APD_{90} values were plotted versus time. The plots were then fitted using the software SigmaPlot 4.0 (SPSS Inc.) with monoexponential equations for increasing PCL protocols: \( y = y_0 + a(1 - \exp(-bx)) \) and biexponential equations for decreasing PCL protocols: \( y = y_0 + a\exp(-bx) + c\exp(-dx) \). Time-constants (\( \tau = 1/b \) and \( \tau' = 1/d \)) obtained from both groups of dogs were then compared.

**Statistical analysis.** Data are expressed as mean ± SEM. Statistical analysis was performed with the Student \( t \) test or with the one-way or two-way analysis of variance completed by a \( t \) test for multiple comparisons (Bonferroni procedure) when adequate. Statistical significance was set at \( p < 0.05 \).

**RESULTS**

**In vivo studies.** In control conditions, HR and QT interval duration were similar in affected and unaffected dogs (respectively, 106 ± 4 vs. 96 ± 8 beats/min for HR, NS and 206 ± 8 vs. 216 ± 6 ms for QT interval, NS). In contrast, the PR interval was longer in affected dogs (117 ± 7 ms) than it was in unaffected dogs (99 ± 3 ms, \( p < 0.05 \)).

Effects of dl-sotalol were assessed after beta-blockade with atenolol. Atenolol decreased HR and increased PR and QT intervals significantly and similarly in both groups. Dl-sotalol further decreased HR (Fig. 1A) similarly in both groups and had no effect on PR interval (Fig. 1B). It prolonged the QT interval in both groups (Fig. 1C) but more in affected dogs than in unaffected ones (15 min after the second injection of dl-sotalol; 31 ± 5 ms and 14 ± 3 ms respectively, \( p < 0.05 \)). None of the unaffected dogs displayed arrhythmias during these experiments. Arrhythmic events occurring in affected dogs are summarized in Table 1.

**In vitro studies.** Electrophysiological characteristics of endomyocardial biopsies and Purkinje fibers in control conditions. Table 2 summarizes the control electrophysiological characteristics of endomyocardial biopsies and Purkinje fibers obtained from unaffected and affected dogs at steady-state (PCL = 1,000 ms). Resting potential, action potential amplitude, maximum upstroke velocity of phase 0 and phase 1 amplitude were similar in both groups. In contrast, APD of biopsies and Purkinje fibers was significantly longer in affected dogs than it was in unaffected ones, especially at long PCL (Fig. 2) (Fig. 5 [12] for Purkinje fibers). This APD prolongation was much greater on Purkinje fibers (Table 2). In control conditions, when PCL increased from 1,000 ms to 8,000 ms, APD_{90} increased by 85% in affected dogs (from 358 ± 16 ms to 664 ± 72 ms) and by only 60% in unaffected dogs (from 302 ± 9 ms to 484 ± 27 ms). Consequently, at long PCL, EADs were observed in 41% of Purkinje fibers of affected dogs (and TA in 18%) but in none of the Purkinje fibers of unaffected dogs.

We focused our study of the kinetics of adaptation of APD_{90} on slow changes described by Boyett et al. (22) on canine Purkinje and ventricular fibers. In controlled dogs, time-constants in myocardium were lower than they were in
Abnormal \( I_\text{Kr} \) in Dogs With Inherited Sudden Death

Mérot et al.

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September 2000:939–47

Table 3. Time Constants of Adaptation of the APD\(_{90}\) to a Change in Pacing Cycle Length on Endomyocardial Biopsies and Purkinje Fibers in Control Conditions

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Endomyocardial Biopsies</th>
<th>Purkinje Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unaffected (4 Dogs, 40 ± 2 Weeks)</td>
<td>Affecte (4 Dogs, 35 ± 3 Weeks)</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 7</td>
</tr>
<tr>
<td>300–1,000 ms</td>
<td>45 ± 5</td>
<td>31 ± 3*</td>
</tr>
<tr>
<td>tau (s)</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>1,000–8,000 ms</td>
<td>44 ± 7</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>tau (s)</td>
<td>n = 5</td>
<td>n = 6</td>
</tr>
<tr>
<td>8,000–1,000 ms</td>
<td>1.7 ± 1.3</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>tau (s)</td>
<td>30 ± 10</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>1,000–300 ms</td>
<td>n = 5</td>
<td>n = 6</td>
</tr>
<tr>
<td>tau (s)</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>tau(_{2}^\prime)</td>
<td>18 ± 1</td>
<td>13 ± 2(_{2}^\prime)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, rounded off to nearest whole number. The number of dogs and their mean age are given in parentheses. The number of experiments is given in italics. See protocol in Methods section.

tau and tau\(_{2}^\prime\) = means of time-constants measured in all experiments.

\( p < 0.05 \) versus unaffected; \( p < 0.05 \) versus endomyocardium.

Purkinje fibers, suggesting that the adaptation of APD\(_{90}\) is faster on myocardium as previously described by Robinson et al. (23). In affected dogs, time-constants were about 30% lower than they were in unaffected dogs on both tissues and for all protocols (Table 3), which means that the adaptation of APD\(_{90}\) to a decrease or an increase in PCL was faster in affected dogs than it was in unaffected ones (Fig. 3 for an affected dog, d,l-sotalol 10\(^{-5}\) M even induced EADs in 4/7 biopsies in affected dogs. On Purkinje fibers in control conditions, EADs were not observed in the unaffected group, whereas 1/5 fibers exhibited EADs in the affected group. In the presence of E-4031 10\(^{-5}\) M, 5/9 Purkinje fibers of unaffected dogs displayed EADs or TA at PCL = 8,000 ms, versus 3/5 fibers of affected dogs in the same conditions.

Cellular electrophysiological effects of chromanol 293B. As shown in Table 4 and Figure 6, 293B slightly but significantly prolonged AP on both endomyocardial biopsies and Purkinje fibers in unaffected dogs but had no effect in affected dogs. After superfusion with 293B 10\(^{-5}\) M, there was no more difference in endomyocardial APD\(_{90}\) between both groups of dogs. 293B had no effect on other AP parameters.

DISCUSSION

In control conditions on endomyocardial biopsies, EADs were not observed in any groups of dogs. E-4031 10\(^{-6}\)M induced EADs in 5/6 samples in the unaffected group and in 4/7 biopsies in affected dogs. On Purkinje fibers in control conditions, EADs were not observed in the unaffected group, whereas 1/5 fibers exhibited EADs in the affected group. In the presence of E-4031 10\(^{-5}\)M, 5/9 Purkinje fibers of unaffected dogs displayed EADs or TA at PCL = 8,000 ms, versus 3/5 fibers of affected dogs in the same conditions.

In previous studies (9,10,18), the phenotype of German shepherds predisposed to sudden death has been characterized. Dogs with strong phenotype exhibit rapid ventricular tachyarrhythmias usually preceded by a sinus pause or bradycardia. Consistently, the arrhythmias disappear during sinus tachycardia. The most severe arrhythmias resemble 'torsade de pointes,' the arrhythmias occurring in patients with long QT syndrome. However, affected dogs do not have a long QT interval, although they present frequent
notching of the T-wave. Moreover, arrhythmias are often associated with variations of the T-wave morphology, suggesting they could depend on abnormalities of repolarization.

**Abnormal cardiac repolarization.** D,l-sotalol is a beta-blocker with class III antiarrhythmic properties (15,24). This drug is known to cause severe cardiac arrhythmias in patients or animal models with prolonged QT interval (25,26) but also in patients with otherwise normal QT interval (25). In vitro, it was shown that d,l-sotalol prolongs AP mainly through a block of the delayed rectifier potassium current I_K (27) and can induce EADs (28). In this study in control conditions, QT interval was similar in both unaffected and affected dogs. But affected dogs presented a greater increase in QT interval than unaffected ones. Consistently, the incidence and severity of arrhythmias increased after d,l-sotalol injection. D,l-sotalol had no proarrhythmic effect in unaffected dogs. In vitro in control conditions, AP recorded from endomyocardium and Purkinje fibers are longer in affected dogs than they are in unaffected ones. This difference is more pronounced as PCL lengthens, with the occurrence of EADs and TA in affected dogs. This could explain the bradycardia dependence of ventricular arrhythmias observed in vivo. Consistently in vitro, the increase in APD on endomyocardium and Purkinje fibers induced by d,l-sotalol was greater in affected dogs leading to the occurrence of EADs and TA on Purkinje fibers and in one endomycocardial biopsy, confirming that this abnormality could be the trigger of the arrhythmias in vivo (11,12).

Our results also show that the adaptation of APD_90 to changes in PCL is faster in affected dogs. In other words in affected dogs when HR slows down, APD would lengthen more and more rapidly. But because sinus variability is large in dogs, there is no available effective method to perform computer analysis of the QT/RR relationship as it is done in humans, so we can only speculate that the QT adaptation to changes in HR would be faster in the affected dogs. This

### Table 4. Effects of D,l-sotalol, E-4031 and Chromanol 293B on Repolarization of Canine Endomyocardial Biopsies and Purkinje Fibers Paced at a Cycle Length of 1,000 ms

<table>
<thead>
<tr>
<th></th>
<th>D,l-sotalol</th>
<th>E-4031</th>
<th>Chromanol 293B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biopsies APD_90 (ms)</td>
<td>Biopsies APD_90 (ms)</td>
<td>Biopsies APD_90 (ms)</td>
</tr>
<tr>
<td></td>
<td>Purkinje APD_90 (ms)</td>
<td>Purkinje APD_90 (ms)</td>
<td>Purkinje APD_90 (ms)</td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(n = 7, 6 dogs, 34 ± 4 weeks)</td>
<td>(n = 6, 5 dogs, 44 ± 2 weeks)</td>
<td>(n = 6, 5 dogs, 45 ± 14 weeks)</td>
</tr>
<tr>
<td>First dose</td>
<td>188 ± 8</td>
<td>265 ± 19</td>
<td>204 ± 5</td>
</tr>
<tr>
<td>Second dose</td>
<td>193 ± 7</td>
<td>299 ± 21</td>
<td>208 ± 5</td>
</tr>
<tr>
<td></td>
<td>217 ± 8*</td>
<td>398 ± 33*</td>
<td>210 ± 5*</td>
</tr>
<tr>
<td><strong>Affected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(n = 8, 6 dogs, 41 ± 5 weeks)</td>
<td>(n = 6, 7 dogs, 50 ± 2 weeks)</td>
<td>(n = 5, 5 dogs, 55 ± 10 weeks)</td>
</tr>
<tr>
<td>First dose</td>
<td>203 ± 6</td>
<td>304 ± 7</td>
<td>209 ± 6</td>
</tr>
<tr>
<td>Second dose</td>
<td>220 ± 7*</td>
<td>345 ± 9</td>
<td>208 ± 4</td>
</tr>
<tr>
<td></td>
<td>250 ± 10*</td>
<td>451 ± 24*</td>
<td>209 ± 5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, rounded off to nearest whole number. The number of experiments, the number of dogs and their mean age are given in parentheses, respectively.

**APD_90** = action potential duration at 90% of full repolarization respectively; first dose = first concentration of each drug, respectively 10^-5, 10^-7 and 10^-8 M for d,l-sotalol, E-4031 and chromanol 293B; second dose = second concentration of each drug, respectively 10^-4, 10^-6, and 10^-5 M for d,l-sotalol, E-4031 and chromanol 293B.

*p < 0.05 versus control.
could be an important arrhythmogenic factor. Indeed, increased variability of QT interval duration was observed in many human cardiac diseases and seems to be a good marker of arrhythmogenicity (7,29). This faster adaptation of QT interval to changes in HR, often called “repolarization lability” (30), agrees with an alteration of repolarization of QT interval to changes in HR, often called “repolarization lability” (30), agrees with an alteration of repolarization dynamics and could facilitate arrhythmias in affected dogs.

Abnormal IKs? Previous patch-clamp studies have shown a decreased I_{to} in epicardial myocytes of affected dogs. This was proposed as the mechanism of AP prolongation (31). However, a specific block of I_{to} in vitro leads to a decrease in APD on canine Purkinje fibers (32), which is not in agreement with our results. Moreover, in ex vivo experiments on canine ventricular samples, blocking I_{to} decreased endocardial APD and diminished the notch of the epicardial AP and the J-wave on ECG, without occurrence of arrhythmias (33). Consequently, a reduced I_{to} cannot explain all the abnormalities of cardiac repolarization observed in this model.

We, therefore, decided to characterize more precisely the late phase of repolarization of affected dogs. We studied the effects of specific blockers of the two components of IK: E-4031 is a class III antiarrhythmic compound that specifically blocks the rapid component of the delayed rectifier current IKr (16,34). In agreement with previous studies (35), we observed a major increase in APD caused by E-4031 on both endomyocardial biopsies and Purkinje fibers. This increase was similar in both groups of dogs, suggesting that IKr may not be implicated in the abnormal cardiac repolarization displayed by affected dogs.

Chromanol 293B is a specific IKs-blocker (17). As previously shown (36), we observed that chromanol 293B slightly but significantly increased APD in unaffected dogs, suggesting that IKs normally participates to cardiac repolarization (36,37). In contrast, chromanol 293B had no effect

Figure 4. Left panels. Increase in APD_{90} induced by dl-sotalol 10^{-5} M (open symbols) and 10^{-4} M (solid symbols) as a function of PCL in (A) endomyocardial biopsies of unaffected (n = 7; circle) and affected dogs (n = 8; triangle; #, n = 7) and (B) Purkinje fibers of unaffected (n = 6; circle) and affected dogs (n = 5; triangle). In the affected group, the number of experimental data decreased to 3 at PCL = 4,000 ms under sotalol 10^{-5} and 10^{-4} M and to 4 at PCL = 2,000 ms under sotalol 10^{-4} M because of the occurrence of early afterdepolarizations or triggered activity. Right panels. (C) Typical action potentials recorded in endomyocardial biopsies of an unaffected dogs (bottom) and an affected dog (top) under control conditions (C) and in presence of dl-sotalol 10^{-5} M (□) and 10^{-4} M (◇) at PCL = 1,000 ms. Vertical bar, 50 mV; horizontal bar, 100 ms. (D) Typical action potentials recorded from Purkinje fibers of an unaffected dog (bottom) and an affected dog (top) in the presence of dl-sotalol 10^{-4} M at PCL = 8,000 ms. Vertical bar, 50 mV; horizontal bar, 2 s. APD = action potential duration; PCL = pacing cycle length.

Figure 5. Left panels. Increase in APD_{90} induced by E-4031 10^{-7} M (open symbols) and 10^{-6} M (solid symbols) as a function of PCL in (A) endomyocardial biopsies of unaffected (n = 6; circle) and affected dogs (n = 7; triangle) and (B) Purkinje fibers of unaffected (n = 9; circle) and affected dogs (n = 5; triangle). At PCL = 4,000 ms, the number of experimental data decreased to 4 under E-4031 10^{-7} and 10^{-6} M in the unaffected group and to 4 under E-4031 10^{-7} M and 2 under 10^{-6} M in the affected group because of the occurrence of early afterdepolarizations or triggered activity. Right panels. Typical action potentials recorded in (C) endomyocardial biopsies and (D) Purkinje fibers of affected dogs (top) and unaffected dogs (bottom) under control conditions (C) and in the presence of E-4031 10^{-7} M (□) for Purkinje fibers only) and 10^{-6} M (◇) at PCL = 1,000 ms. Vertical bar, 50 mV; horizontal bars, 100 ms in (C) and 200 ms in (D). APD = action potential duration; PCL = pacing cycle length.
Abnormal \( I_{Ks} \) in Dogs With Inherited Sudden Death

Mechanisms for an altered \( I_{Ks} \). To explain an abnormal \( I_{Ks} \), we can either hypothesize a mutation on genes encoding for the proteins supporting this ionic current or a dysfunction in its maturation process. The \( I_{Ks} \) current is formed by the coassembly of two proteins, \( KvLQT1 \) (the channel) and \( I_{sK} \) (the regulator), encoded respectively by the \( KCNQ1 \) and \( KCNE1 \) genes (38). Mutations on these genes are responsible for two different long QT syndromes in humans, respectively LQT1 (39) and LQT5 (40). The abnormality observed in affected dogs of this genetic model could, therefore, be due to a mutation on either \( KCNQ1 \) or \( KCNE1 \). But it can also be linked to an abnormal expression of one of these two genes mediated by the autonomic nervous system (ANS). Indeed, ANS stimulation and development may play a role in the genesis of arrhythmias (1,7,41). Neuromediators of ANS are suggested to play a role in the maturation of cardiac cells and molecular structures, especially ionic channels. In vitro experiments using neuromediators or hormones can modify the expression of ionic channels (42,43). Abnormal development of ANS may, therefore, lead to abnormal expression of ionic channels (7).

Dae et al. (44) showed that affected dogs have a heterogeneous cardiac sympathetic innervation. This could lead to an abnormal expression of genes encoding for ionic channels in affected dogs, and it explains the decreased \( I_{to} \) observed on epicardial myocytes (31) and the decreased \( I_{Ks} \) suggested by our results on endomyocardium and Purkinje fibers. The prolongation of PR interval may also be due to this heterogeneous innervation and may implicate an abnormal expression of genes encoding for sodium or calcium channels.

Conclusions. We conclude that abnormal repolarization might be the cause of arrhythmias in this canine genetic syndrome. This abnormality may be due to an abnormal sympathetic innervation and may implicate dysfunctions of the potassium channel responsible for \( I_{Ks} \). This would lead to electrogenic instability of cardiac tissue responsible for the prolongation of repolarization on myocardium and Purkinje fibers, the occurrence of EADs and TA on Purkinje fibers and the faster kinetics of adaptation of repolarization to changes in cardiac rate. Further investigations are expected to confirm a mutation or an abnormal expression of genes encoding for ionic channels.

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