Age dependence of the development of ventricular arrhythmias in a canine model of sudden cardiac death

N. Sydney Moïse a,*, Mark L. Riccio a,b, Bruce Kornreich a, William J. Flahive Jr. a, Robert F. Gilmour Jr. b

a Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, USA
b Department of Physiology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, USA

Received 6 September 1996; accepted 4 February 1997

Abstract

Objectives: The age-dependence of the development of ventricular arrhythmias was studied in German shepherd dogs with inherited ventricular arrhythmias and sudden death. Background: A colony of German shepherd dogs has been established that exhibit inherited ventricular arrhythmias and sudden death. The incidence of arrhythmias increases with age. Because ventricular tachycardia is associated with bradycardia, it was hypothesized that the increased incidence of arrhythmias was related to age-dependent slowing of heart rate.

Methods: Arrhythmia counts and RR intervals were measured from serial ambulatory ECG recordings obtained in 71 dogs 1±48 weeks. In addition, 19 dogs were challenged with phenylephrine 10 mg/kg i.v. at 15, 28, and 45 weeks of age, 10 dogs were challenged with epinephrine (1 µg/kg i.v.) at 3, 5, 7, 9, 11, 18, and 28 weeks of age, and 10 dogs were challenged at 28 weeks with epinephrine (2.5 µg/kg i.v.), before and after propranolol (0.5 mg/kg i.v.). Results: The incidence and severity of ventricular arrhythmias increased between 7 and 28 weeks of age and decreased between 28 and 44 weeks of age. The age-dependent increase in the incidence of ventricular tachycardia was associated with age-dependent reductions in sinus rate. Baroreflex-mediated slowing of the heart rate unmasked arrhythmias in young animals that did not spontaneously display arrhythmias and exacerbated existing arrhythmias in older animals. However, the magnitude of baroreflex-induced bradycardia was similar from 7±18 weeks of age, yet the incidence of arrhythmias increased progressively. Moreover, the waning of ventricular arrhythmias in older animals was not associated with more rapid sinus rates. Conclusion: The risk for sudden death in dogs with inherited ventricular arrhythmias increases with age in part because of age-dependent slowing of heart rate and in part because of other heart-rate-independent factors. The correspondence between the development of ventricular tachycardia and sinus pauses is consistent with the hypothesis that ventricular arrhythmias are initiated by early afterdepolarization-induced triggered activity.

Keywords: Sudden death; Arrhythmias; Sympathetic nervous system; Heart rate; Dog, anesthetized

1. Introduction

Animal models of spontaneous ventricular arrhythmias and sudden cardiac death are rare [1]. To better understand the mechanisms that predispose to sudden death, we have established a colony of German shepherd dogs that exhibit inherited ventricular arrhythmias and sudden arrhythmic death [2]. Although the specific substrate responsible for the spontaneous arrhythmias in these dogs is unknown, certain factors such as heart rate, behavior and time of day influence the expression of the arrhythmia and death in these animals [3]. In addition, preliminary observations have suggested that the development of the arrhythmias is age-dependent [2]. The incidence of ventricular arrhythmias increases from birth to 4–8 months of age, at which time the incidence of sudden death is highest [2].

In the present study serial 24-h electrocardiographic monitoring was used to document that the incidence of ventricular arrhythmias in this group of German shepherd dogs was age-dependent. We also investigated possible
mechanisms for the age-dependence. Given that our previous studies have indicated that the arrhythmias in these animals typically are associated with sinus bradycardia [2,3], we tested whether the increased incidence of arrhythmias with increasing age was associated with age-dependent reductions in heart rate. In addition, we tested whether baroreflex-mediated slowing of the heart rate unmasked arrhythmias in young animals that did not spontaneously display arrhythmias and exacerbated arrhythmias in older animals in whom arrhythmias were present. Finally, we tested whether the waning of ventricular arrhythmias in some older animals was associated with decreased baroreflex-mediated sinus bradycardia.

2. Methods

2.1. Animals

Animals used in this study were housed in an American Association for the Accreditation of Laboratory Animal Care approved facility that conforms to the guidelines for proper animal care, as described within the NIH Guide for the Care and Use of Laboratory Animals and the position of the American Heart Association on Research Animal Use. Experiments were approved by the Institutional Animal Care and Use Committee of the Center for Research Animal Resources at Cornell University. All dogs were obtained from a colony of purebred German shepherd dogs with inherited ventricular arrhythmias and a predisposition to sudden death.

2.2. Ambulatory electrocardiographic monitoring

To determine whether the development of ventricular arrhythmias in this colony of dogs was age-dependent, serial ambulatory ECG monitoring was performed in 71 dogs. Twenty-four-hour ECG recordings were obtained monthly between 8 and 26 weeks of age. Nineteen of these dogs were monitored further at 3–5-week intervals until at least 35 weeks of age. Additionally, in 12 of the 71 dogs, 2-h ambulatory ECG recordings were obtained between 1 and 7 weeks of age.

The RR intervals from the ambulatory ECG recordings were analyzed using a computer program based on algorithms described by Pan and Tompkins [4]. The program was written using MATLAB 4.2c (The Mathworks, Inc., Natick, MA) and run on a Power Macintosh 8100/80 computer (Apple Computer, Inc., Cupertino, CA). First return plots (Poincaré plots) of the RR intervals were generated by plotting $RR_n$ versus $RR_{n+1}$.

Because of the polymorphic configuration of the ventricular arrhythmias, short coupling intervals (frequently less than 200 ms), large T-waves, high sinus heart rate and the rapid rates of the non-sustained (less than 30 s) runs of ventricular tachycardia (greater than 400 beats/min), characterization of ventricular arrhythmias using automated analysis of the recordings was not possible. Consequently, all recordings were analyzed by visual inspection by individuals experienced in the interpretation of the canine ECG. All premature complexes were manually counted, not analyzed by computer. Premature ventricular complexes (PVCs) were counted and categorized into the following groups: multiple PVCs, defined as the total number of PVCs encompassed by couplets, triplets, and ventricular tachycardia; ventricular tachycardia, defined as 4 or more PVCs in a row; total premature ventricular complexes, defined as the sum of multiple PVCs and single PVCs. Previous studies have shown that severely affected animals frequently have in excess of 50 000 PVCs per 24-h period [2]. To facilitate data analysis, therefore, the maximum number of ventricular complexes counted during any 24-h monitoring period was limited to 5000 for each of the categories. For certain analyses, each dog’s arrhythmia counts for a given category were normalized by dividing the arrhythmia counts by the maximum number of counts within that category.

To determine whether the development of ventricular tachycardia was associated with sinus bradycardia or pauses, RR intervals preceding ventricular tachycardia were analyzed from the last 24-h recording before sudden death in 10 dogs. RR intervals were measured during the 20 s preceding each of 10 runs of ventricular tachycardia. In each dog, the longest runs of ventricular tachycardia (greater than 8 consecutive ventricular complexes) that occurred between 01.00 and 08.00 h were analyzed. This time period was selected because most of the animals died during this time of day. None of the dogs was wearing a monitor at the time of death.

2.3. Phenylephrine and epinephrine challenge

To determine whether baroreflex-mediated slowing of the heart rate induced arrhythmias in young animals that did not spontaneously display arrhythmias and exacerbated arrhythmias in older animals in whom arrhythmias were present, 19 anesthetized dogs were challenged with phenylephrine (10 µg/kg, given as an i.v. intravenous bolus) at 15, 28, and 45 weeks of age. A separate group of 10 dogs was challenged with epinephrine (1 µg/kg, given as an i.v. bolus) at 3, 5, 7, 9, 11, 18, and 28 weeks of age. Ten additional dogs were challenged at 28 weeks with epinephrine (2.5 µg/kg i.v.), before and after treatment with propranolol (0.5 mg/kg i.v.). Challenges were made during a constant fentanyl infusion at a rate of 0.04 mg/kg/h.

Analog signals were amplified using a Gould ES 2000 (Gould, Inc., East Rutherford, NJ) and the amplified signals were digitized using a data acquisition system (BioPac Systems, Goleta, CA) and a Power Macintosh 7100/66AV (Apple Computer, Inc., Cupertino, CA). Each channel was sampled at 200 Hz with 12-bit resolution and recorded to
the internal hard drive for the duration of the experiment. Arrhythmia counts were determined for the 6 min preceding either phenylephrine or epinephrine administration and for 6 min after drug administration.

The average RR intervals before and after treatment with phenylephrine, epinephrine, propranolol, and epinephrine plus propranolol were determined by measuring RR intervals for 15 s during the last minute before drug administration and for 15 s during the peak arrhythmogenic effect, which occurred between 1 and 3 min after drug administration. In dogs that did not develop arrhythmias after phenylephrine or epinephrine, the average RR interval was determined for 15 s beginning 2 min after drug administration. When ventricular arrhythmias were present, RR intervals were measured between consecutive sinus and ventricular escape complexes and between PVCs and sinus or escape complexes. The RR intervals of multiple PVCs were not included in these measurements. The purpose of this evaluation was to document the increased RR intervals with α1-adrenergic agonist treatment.

2.4. Statistical analysis

Data are presented as means ± standard error. Comparisons before and after treatments were made using a paired t-test or an analysis of variance with a Fisher’s PLSD correction for multiple comparisons.

3. Results

3.1. Age-dependence of ventricular arrhythmias during 24-h ambulatory electrocardiographic monitoring

Of the 71 dogs studied, 19 had less than 10 PVCs on any 24-h ECG recording. In our experience with canine recordings this infrequent number of PVCs is within normal variation. Thus, these animals were defined as unaffected and were not analyzed further because the purpose of this study was to determine the development of arrhythmias in affected dogs. The remaining 52 dogs displayed a large variation in the frequency of the ventricular arrhythmias, ranging from 10 single PVCs/h to greater than 10 runs/h of rapid ventricular tachycardia. The appearance of spontaneously occurring arrhythmias was age-dependent, in that 49 of 52 dogs (94%) had fewer than 10 PVCs/24 h between 8 and 11 weeks of age, but only 1 of 48 dogs (2%) had fewer than 10 PVCs/24 h at 23–28 weeks of age.

Only 19 of the 52 dogs were followed for the extended time of at least 35 weeks because the other animals were used for other terminal experiments [5–9]. However, these 19 dogs represented the full phenotypic spectrum of affectedness (few to many ventricular arrhythmias except none of these dogs died suddenly). The incidence of arrhythmias in these dogs was low at 8–11 weeks and increased in all dogs to a maximum at approximately 22–26 weeks. Thereafter, arrhythmia incidence declined in 12 dogs and remained the same or increased in 7 dogs, so that by 33–45 weeks of age mean values for total premature ventricular complexes, multiple PVCs and ventricular tachycardia were not significantly different from those observed at 8–11 weeks of age (Fig. 1).

3.2. Association between sinus pauses and the onset of ventricular tachycardia

Because our previous studies had provided anecdotal evidence that the onset of ventricular tachycardia in these dogs frequently is preceded by a sinus pause [2,3], we tested whether, in fact, this relationship existed by analyzing the RR intervals that preceded the onset of ventricular tachycardia in 10 of the 52 affected dogs that subsequently died suddenly. Ventricular tachycardia was more likely to be preceded by a long RR interval than by a short RR interval. The mean (± s.e.m.) RR interval preceding a run of ventricular tachycardia was 1130.7 ± 37.4 ms (n = 100),
whereas the RR interval preceding a single PVC was 819.8 ± 14.3 ms (n = 623) (P < 0.001).

3.3. Age-dependence of RR intervals

Given our observation that ventricular tachycardia was more likely to be preceded by a long RR interval than by a short RR interval, we next tested whether the increased incidence of ventricular arrhythmias between 1 and 25 weeks of age was associated with slowing of the heart rate. We also tested whether the decline of ventricular arrhythmias in some dogs between 25 and 45 weeks of age was associated with an increase in heart rate.

---

**Fig. 2.** First return plots of RR intervals for an unaffected (left panels) and an affected (right panels) German shepherd dog at the ages indicated. With increasing age, the range of RR intervals increased in both dogs. Ventricular arrhythmias, consisting primarily of PVCs, were first observed at 15 weeks of age in the affected dog (as indicated by the dark bands of RR250±300 ms; see arrows). At 45 weeks of age, ventricular arrhythmias, consisting of PVCs and ventricular tachycardia, persisted in the affected dog. See text for further discussion.
Mean RR interval increased slightly, but significantly, between 1 (affected 263.2 ± 9.8, unaffected 270.3 ± 9.6 ms) and 4 (affected 291.7 ± 9.7, unaffected 293.7 ± 10.4 ms) weeks of age, and decreased between 4 and 7 (affected 269.6 ± 7.7, unaffected 260.8 ± 9.4 ms) weeks of age. Thereafter, mean RR interval increased significantly to 24 (affected 454.2 ± 56.8, unaffected 470.2 ± 14.3 ms) weeks of age. There was no significant difference between affected and unaffected dogs in the age-dependent changes in heart rate over this range of ages. Meaningful determi-

Fig. 3. First return plots of RR intervals for an affected German shepherd dog at 7, 11, 24, 28 and 45 weeks of age. At 7 weeks of age, RR intervals were restricted primarily to a range of 300–400 ms and there were no PVCs. The upper end of the range of sinus RR intervals increased between 7 and 11 weeks of age. Single PVCs were first seen at 11 weeks of age, as indicated by the cluster of short RR intervals (arrow in lower left panel). PVCs typically followed RR intervals of 400–600 ms and were not observed after longer or shorter RR intervals. At 24–28 weeks of age the upper end of the range of RR intervals was increased further and PVCs occurred after both long and short RR intervals, as indicated by the dark bands of RRn,1 = 250–300 ms. At 45 weeks of age, ventricular arrhythmias had subsided (open arrow), despite a further increase in the upper end of the range of RR intervals. See text for further discussion.
nations of sinus rate in affected dogs was difficult at older ages because of the high incidence of ventricular arrhythmias in some dogs. However, the RR interval continued to increase in unaffected dogs to 35 weeks of age (652.7 ± 56.7 ms).

The first ventricular arrhythmias to occur in these dogs were single PVCs, which, somewhat surprisingly, were preceded by relatively short RR intervals (Figs. 2 and 3). With maturation, the upper end of the range of RR intervals increased, as did the range of RR intervals that were followed by a PVC (Figs. 2 and 3). However, in agreement with the results shown above, ventricular tachycardia was more likely to occur after a long, rather than a short, RR interval. There was no reduction in the incidence of long

Fig. 4. Age-dependent effects of phenylephrine on the incidence of ventricular arrhythmias in affected dogs. Total premature ventricular complexes, multiple PVCs and ventricular tachycardia were counted before and after phenylephrine administration (10 μg/kg i.v. bolus) at 15, 28 and 45 weeks of age. Different symbols represent different dogs. See text for discussion.
RR intervals in those dogs in whom arrhythmias eventually subsided, as illustrated by the example shown in Fig. 3.

### 3.4. Effects of phenylephrine and epinephrine on ventricular arrhythmias

The results shown above suggested that the incidence of ventricular tachycardia increased with age because of an age-dependent increase in the incidence of sinus pauses. This result further suggested that ventricular arrhythmias might be unmasked in young animals by slowing the heart rate. To test these hypotheses, the age-dependence of the effects of phenylephrine on the development of ventricular arrhythmias was analyzed in 19 affected animals.

Phenylephrine significantly increased total premature ventricular complexes and multiple PVCs at 15 weeks of age and significantly increased total premature ventricular complexes at 28 weeks of age (Fig. 4). At 45 weeks of age, phenylephrine did not increase the overall incidence of ventricular arrhythmias significantly (Fig. 4). At this age 9 of the 19 dogs had no arrhythmias at baseline. Four of these dogs developed arrhythmias after phenylephrine and each of the 4 dogs had arrhythmias detected by 24-h ECG monitoring at this age. However, 5 dogs did not have arrhythmias induced by phenylephrine and had no arrhythmias detected on 24-h ECG monitoring, although each of these animals had displayed arrhythmias at a younger age. Of the 10 dogs that displayed arrhythmias at baseline, phenylephrine increased total premature ventricular complexes in 8 dogs and decreased total premature ventricular complexes in the remaining 2 dogs. Total premature ventricular complexes, multiple PVCs and ventricular tachycardia after phenylephrine treatment were not significantly different between ages. RR intervals at baseline increased significantly between 15 and 28 weeks of age and between 28 and 45 weeks of age and were increased further after administration of phenylephrine at all ages (Table 1).

The age-dependence of the effects of epinephrine on the development of ventricular arrhythmias was analyzed in a separate group of 10 affected animals. Two dogs were

### Table 1

<table>
<thead>
<tr>
<th>Age in Weeks</th>
<th>Baseline RR Interval</th>
<th>Treatment RR Interval</th>
<th>Baseline vs Treatment P Value</th>
<th>Percent Increase in Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>15</td>
<td>780 ± 26</td>
<td>1168 ± 56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td>28</td>
<td>1104 ± 73</td>
<td>1371 ± 137</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>45</td>
<td>1242 ± 56</td>
<td>1511 ± 99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>7</td>
<td>575 ± 38</td>
<td>942 ± 78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>9</td>
<td>603 ± 41</td>
<td>1026 ± 73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>11</td>
<td>817 ± 51</td>
<td>1060 ± 46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>838 ± 65</td>
<td>955 ± 77</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1063 ± 82</td>
<td>1213 ± 95</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Mean ± standard error.

### Table 2

<table>
<thead>
<tr>
<th>Total Arhythmias (PVCs/min)</th>
<th>Multiple PVCs (PVCs/min)</th>
<th>Ventricular Tachycardia (PVCs/min)</th>
<th>RR Interval (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>18.0 ± 13.0</td>
<td>14.9 ± 12.0</td>
<td>12.1 ± 11.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1125 ± 1114</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>31.4 ± 19.7</td>
<td>p&lt;0.01</td>
<td>28.0 ± 19.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td>19.6 ± 18.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1074 ± 92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Epinephrine + Propranolol</td>
<td>50.4 ± 20.4</td>
<td>p&lt;0.05</td>
<td>44.5 ± 21.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td>33.2 ± 20.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1598 ± 150</td>
</tr>
</tbody>
</table>

* Mean ± standard error. Differences in arrhythmias and RR intervals between baseline and epinephrine were not significant.
challenged with epinephrine at 3 and 5 weeks of age, but minimal slowing of heart rate occurred (heart rate remained greater than 100 beats/min). Therefore, challenges of the other 8 dogs were delayed until 7 weeks of age. During anesthesia, baseline arrhythmias were absent at 7 and 9 weeks, first appeared at 11 weeks and thereafter increased significantly to 28 weeks of age (Fig. 5). After epinephrine, arrhythmias were induced as early as 7 weeks of age, with total premature ventricular complexes increasing significantly at 7, 11, and 18 weeks and approaching significance at 9 weeks of age (Fig. 5). Baseline RR intervals increased between 7 and 18 weeks of age and were increased further by epinephrine over this range of ages (Table 1). In contrast, at 28 weeks of age epinephrine did not significantly alter total premature ventricular complexes, multiple PVCs, ventricular tachycardia or the RR interval (Fig. 5 and Table 1). However, because of the age-related increase in the baseline RR interval at 28 weeks, the RR interval after epinephrine at this age was not significantly different from the RR intervals after epinephrine at the younger ages (Table 1).

Total premature ventricular complexes were significantly increased at 28 weeks of age with phenylephrine, but not with epinephrine. To determine whether the β-

![Fig. 5. Age-dependent effects of epinephrine on the incidence of ventricular arrhythmias in affected dogs. Total premature ventricular complexes, multiple PVCs and ventricular tachycardia were counted before and after epinephrine administration (1.0 μg/kg i.v. bolus) at 7, 9, 11, 18 and 28 weeks of age. Different symbols represent different dogs. See text for discussion. Dashed lines indicate dogs with a decrease in arrhythmias after treatment.](https://academic.oup.com/cardiovascres/article-abstract/34/3/483/264773)
adrenergic effect of epinephrine influenced the development of arrhythmias at this age, a separate group of affected dogs was challenged at 28 weeks of age with epinephrine before and after treatment with propranolol. In this group of dogs, the incidence of ventricular arrhythmias was not increased after epinephrine alone, but was increased after epinephrine in the presence of propranolol (Table 2). In addition, epinephrine given after pretreatment with propranolol caused ventricular tachycardia to become slower and more sustained. The mean RR interval was not different between baseline and epinephrine treatment, but was increased significantly by epinephrine after pretreatment with propranolol (\(P < 0.01\)) (Table 2).

### 4. Discussion

The results of this study indicate that the incidence and severity of ventricular arrhythmias increased with age in German shepherd dogs at risk for sudden death. Although single PVCs occurred after short RR intervals, ventricular tachycardia was more likely to occur after long RR intervals. Accordingly, the age-dependent increase in the incidence of ventricular tachycardia developed in parallel with an increase in long RR intervals. Prolongation of the RR interval by pharmacological induction of reflex bradycardia also increased the incidence of ventricular arrhythmias, even in young dogs in whom arrhythmias were not detected during ambulatory monitoring. In the majority of the dogs the incidence of ventricular arrhythmias decreased between 25 and 45 weeks of age, despite a progressive increase in RR intervals.

#### 4.1. Age-dependence of the development of ventricular arrhythmias and sudden death

Animals in this colony of inbred German shepherd dogs with inherited ventricular arrhythmias are known to die suddenly, usually between 4 and 8 months of age [2]. The dogs used in this study are more likely to die during presumed sleep, a behavior associated with significant slowing of the heart rate [4]. They do not die before 16 weeks of age and uncommonly die after 12 months of age. Therefore, these animals have a window of vulnerability for sudden death. The results of the present study indicate that the range of ages over which sudden death is most likely to occur corresponds to the ages at which the incidence of ventricular tachycardia is highest. However, ventricular arrhythmias can be induced in dogs as young as 7 weeks of age by administration of \(\alpha_{1}\)-adrenergic agonists, suggesting that the substrate for ventricular arrhythmias exists at an early age, but that the development of ventricular tachycardia is prevented by the high heart rate typical of young dogs [10,11]. Attempts to induce arrhythmias in even younger animals were unsuccessful, probably because of inadequate slowing of the heart rate secondary to incomplete maturation of the baroreceptor reflex [10–12].

#### 4.2. Electrophysiological mechanism for the initiation of ventricular tachycardia

The higher incidence of ventricular tachycardia after long RR intervals is consistent with the initiation of such tachycardias by early afterdepolarization (EAD)-induced triggered activity [13,14]. The potential contribution of EAD-induced triggered activity to the development of ventricular arrhythmias in these dogs has been suggested previously by the observation that Purkinje fibers obtained from the left ventricles of affected dogs displayed EAD-induced triggered activity in vitro [5]. In contrast, Purkinje fibers obtained from the right ventricles of affected dogs or from either ventricle of unaffected dogs did not develop EADs or triggered rhythms. Moreover, the presence of PVCs without a pause may still be EAD-initiated, but could indicate other mechanisms including delayed afterdepolarizations. The development of EAD-induced triggered activity occurred in the absence of a prolonged QT interval [2], suggesting that abnormal repolarization was limited to the Purkinje network. Further studies are needed to determine whether longer RR intervals are associated with a higher incidence of ventricular tachycardia in affected dogs at the peak age of affectedness because they induce longer runs of triggered activity or because they create a dispersion of refractoriness that facilitates the development of re-entry.

#### 4.3. Maturation of the autonomic nervous system and the development of ventricular arrhythmias

It seems likely that both normal and abnormal maturation of the autonomic nervous system contributes to the development of ventricular arrhythmias in this group of dogs. With respect to abnormalities of nerve maturation, dogs from this colony have defects in sympathetic innervation, primarily to their left ventricles [9]. Previous studies have shown that imbalances in the sympathetic innervation to the heart can result in lower thresholds for ventricular fibrillation and that the ventricular fibrillation threshold varies with age, in association with maturation of the autonomic nervous system and changes in the QT interval [15]. In our studies, no prolongation of the QT interval was found [2], yet abnormal repolarization occurred in Purkinje tissue obtained from denervated regions of the heart [5]. Moreover, despite a normal QT, the morphology of the T-wave in affected dogs is different from that in unaffected dogs [16]. This result suggests that the absence of a prolonged QT interval does not preclude the existence of potentially lethal abnormalities of repolarization.

Abnormal maturation of sympathetic innervation in affected dogs may have promoted the development of prolonged repolarization and EADs by inhibiting the functional expression of repolarizing ionic currents in the ventricle. This hypothesis is suggested by the observation that the transient outward potassium current (\(I_{o}\)) is reduced in epicardial and Purkinje myocytes isolated from...
the left, but not the right, ventricles of affected dogs [6] and that $I_{to}$ density is restored by in vitro exposure to norepinephrine [7]. $I_{to}$ density is known to increase during development in the dog [8,17], particularly between 10 and 20 weeks of age. Over this range of ages marked changes in the maturation of ventricular sympathetic innervation occur [18,19].

Maturation of sympathetic innervation also might be associated with changes in adrenergic receptor density and affinity that could alter the development of triggered arrhythmias. In this regard, our previous studies have shown that activation of $\alpha_{1}$-adrenergic receptors increases Purkinje fiber action potential duration and facilitates the development of EAD-induced triggered activity [5]. In contrast, activation of $\beta$-adrenergic receptors by epinephrine suppresses EAD-induced triggered activity, secondary to shortening of action potential duration and acceleration of the spontaneous discharge rate of the Purkinje fiber [5]. Perhaps the abatement of arrhythmias in older dogs is related, in part, to an age-related reduction of $\alpha_{1}$-adrenergic receptors [18] and increase of $\beta$-adrenergic receptor responsiveness [11,20–22].

4.4. Summary

We have documented that dogs with an inherited predisposition to sudden death display a developmentally dependent emergence of ventricular arrhythmias. The expression of the arrhythmias is in part triggered by age-dependent slowing of heart rate. However, other factors, such as abnormal maturation of cardiac sympathetic innervation and repolarizing potassium currents, may influence the creation of potentially fatal arrhythmias in this unique model of spontaneous sudden death.

Acknowledgements

We wish to thank the following individuals for assisting with these studies: Darcy Brittain Adin, Matthew T. Antkowiak, Melissa Beall, Naomi Jeanne Brohard, Jennifer N. Collins, Dee A. Dugger, Sabrina Ernst, Mary Ruth Harbeck, Laura Leisberg, Fred R. Levy, Beth A. Lewis, John MacGregor, Todd Nizialek, Emily C. Pershing, Tara Sparks, Kristina R. Vygantas and Joshua L. Weisberg. We also appreciate the generous donations of DelMar Avionics with special thanks to Dr. Raphael Henkin. This work was supported by grant HD23938 from the National Institute of Child Health and Human Development, Bethesda, MD.

References