Heterogeneous Ventricular Repolarization Provides a Substrate for Arrhythmias in a German Shepherd Model of Spontaneous Arrhythmic Death

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Background—German shepherd dogs with inherited arrhythmias and sudden death appear to be a model for catecholamine-dependent ventricular tachycardias in human subjects. We tested the hypothesis that heterogeneity of left ventricular repolarization creates an arrhythmogenic substrate for pause-dependent ventricular tachycardia in these animals.

Methods and Results—We used microelectrode techniques to record action potentials (AP) from midmyocardial sections of anteroseptal, anterobasal, and posterobasal left ventricular (LV) wall of unafflicted and afflicted dogs. There were no differences in AP duration to 90% repolarization (APD) among LV regions in unafflicted dogs. In contrast, in afflicted dogs, there was significant heterogeneity, with the longest APD in anterobasal and shortest in anteroseptal regions. Isoproterenol did not affect repolarization in unafflicted dogs, whereas in afflicted dogs, it shortened APD anterobasally and prolonged APD anteroseptally. We studied the repolarizing currents, $I_{Kr}$ and $I_{Ks}$, in single anterobasal and anterobasal LV myocytes with the use of a whole-cell voltage clamp. There were no differences in $I_{Kr}$ and $I_{Ks}$ between anteroseptal and anterobasal regions in unafflicted dogs, whereas in afflicted dogs, $I_{Kr}$ was smaller anterobasally ($P<0.05$). Isoproterenol produced a more prominent leftward shift in $I_{Ks}$ voltage-dependent activation in anterobasal regions of afflicted than unafflicted dogs.

Conclusions—Spatial heterogeneity in expression and catecholamine responsiveness of $I_{Kr}$ and $I_{Ks}$ results in heterogeneous LV repolarization in afflicted German shepherd dogs, contributing importantly to the arrhythmogenic substrate. (Circulation. 2003;108:1389-1394.)

Key Words: arrhythmia ■ death, sudden ■ electrophysiology ■ ion channels

A German shepherd model of spontaneously occurring ventricular arrhythmias and sudden death manifests similarities to catecholamine-induced tachycardias in human subjects. In this model, pause-dependent arrhythmias contribute to $\approx80\%$ of tachycardias. Their initiation has been attributed to early afterdepolarizations (EADs), inducing triggered activity that originates from left ventricular (LV) Purkinje fibers. However, the substrate contributing to maintenance of this ventricular tachycardia remains unknown. The pause dependence of the arrhythmias as well as the observations that in afflicted dogs (1) the QT interval is not prolonged, (2) LV sympathetic innervation is inhomogeneous, and (3) frequent T-wave notching occurs, raise the possibility that inhomogeneity of LV repolarization may constitute part of the arrhythmogenic substrate. Therefore, we studied action potential duration (APD) and isoproterenol responsiveness in multicellular sections from anterobasal, anteroseptal, and posterobasal LV midmyocardium, as well as epicardium and subendocardium of hearts from afflicted and unafflicted dogs. To elucidate ionic mechanisms of regional heterogeneity in APD, $I_{Kr}$ and $I_{Ks}$, and their responses to isoproterenol were examined as well.

Methods

All experimental procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication No. 85-23).

Holter monitoring was used to identify 28 arrhythmic (afflicted) German shepherd dogs (22 to 32 weeks of age) from a colony bred at Cornell University. In this age range, the expression of lethal arrhythmias is maximal. Arrhythmias included ventricular premature depolarizations and/or ventricular tachycardia (4 or more ventricular complexes in sequence). Twenty age-matched control (unafflicted) German shepherds were obtained from a commercial breeder and were determined to have no spontaneous arrhythmias.
Ca2+ The LAD or its first branch was cannulated and perfused with supplied by the left anterior descending (LAD) artery was dissected.

isoproterenol superfusion. In some experiments, the repolarization to ing test pulses from 0 to of Helopharm). Currents were recorded during 5-second depolariz-

chromanol 293B (a gift of Aventis Pharma) was used. Currents were recorded using 7.4 with NaOH. L-type Ca2+ was studied.

In another set of experiments, IKr blocker, dofetilide (10−6mol/L) application. 15 Cells were discriminated with a 2-tailed protocol. 15 Cells were stored at room temperature in buffer solution. Isolated cells were subjected to frequency dependence of APD was studied at CL = 4000, 2000, 1000, 500, and 300 ms. Steady state was achieved by pacing at 3 minutes for each CL. After control records were obtained, some preparations were superfused with Tyrode’s solution containing 0.3 mmol/L CaCl2 and agitated with

times, and the supernatant from each digestion was centrifuged. Minced tissues were filleted from the anterobasal LV. Preparations were placed in an

Currents were recorded from impalements maintained in a bath (35°C) perfused with modified Tyrode’s solution containing (mmol/L): NaCl 140, KCl 5.4, CaCl2 1.8, MgCl2 1, HEPES 5, and glucose 10. pH was adjusted to 7.4 with NaOH. L-type Ca2+ current was blocked with 10−6 mol/L nisoldipine (a gift of Bayer, Germany). Currents were recorded using a standard whole cell patch-clamp method. The pipette (1 to 3 MΩ) solution contained (mmol/L): aspartic acid 130, KOH 146, NaCl 10, EGTA 5, CaCl2 2, Mg-ATP 2, HEPES 10, with pH adjusted to 7.2 with KOH.

IKr and IKr were discriminated with a 2-tailed protocol.15 Cells were depolarized from a holding potential of −40 mV to +40 mV, repolarized first to 0 mV and then to −40 mV. The duration of each step was 6 seconds. The first repolarizing step to 0 mV generates mainly an IKr tail, whereas the final step to −40 mV results primarily in an IKr tail.15 Measurements were made before and 90 seconds after isoproterenol superfusion. In some experiments, the IKr/IKr blocker chromanol 293B (a gift of Aventis Pharma) was used.

Ikr was examined in myocytes pretreated with a selective IKr blocker, dofetilide (10−6 mol/L) (a gift of Helopharm). Currents were recorded during 5-second depolarizing test pulses from 0 to +70 mV in 10-mV increments and on repolarization to −10 mV. Pulses were applied from holding potential −40 mV at 10-second intervals to ensure deactivation of tail currents. The protocol was performed before and after 2 minutes of isoproterenol (10−7 mol/L) application.

Statistical Analysis
Microelectrode data were analyzed from impalements maintained throughout each experimental protocol. Data are expressed as mean±SEM. Statistical analysis was by 1- or 2-way ANOVA for multiple groups or for repeated measures, with the Bonferroni test when the F value permitted this. When 2 groups were compared, unpaired or paired t tests were used. Significance was determined at P<0.05.

Results
Studies of Isolated Myocardium
Transmural dispersion of repolarization was examined in the anterobasal LV wall, where midmyocardial APD90 in afflicted dogs was the longest, suggesting a prominent transmural gradient of repolarization. In both groups of dogs, APD90 in midmyocardium was significantly longer than either in subepicardial or subendocardial regions, with a parallel upward shift of the curve for afflicted dogs (Figure 1). Accordingly, the expression of a transmural gradient of repolarization across the ventricular wall was about the same in both groups of animals and also similar to that of normal mongrel dogs.16

Given the comparability of differences across the LV wall, all subsequent studies were performed in midmyocardium only.

Action potential parameters other than APD did not differ among the 3 regions of midmyocardium in unafflicted and afflicted dogs. At CL = 4000 ms, maximum diastolic potential ranged from −88±1 to −90±1 mV across groups; AP amplitude from 116±2 to 119±1 mV and Vmax from 248±12 to 267±19 V/s (n = 38 to 55/group, all P > 0.05). Figure 2A shows representative APs recorded in 3 LV midmyocardial regions of 1 unafflicted and 1 afflicted dog at CL = 4000 ms. A notable difference between the two is evident with prominent heterogeneity of repolarization along the midmyocardium in the afflicted and minimum heterogeneity in the unafflicted dog. Whereas APD90 was not significantly different among LV regions in unafflicted dogs at all CLs, in afflicted dogs, and particularly at long CLs, APD90 was the longest in anterobasal and shortest in anteroseptal regions (Figure 2B).

Figure 3A demonstrates that isoproterenol (10−7 mol/L) minimally affected repolarization in an unafflicted dog but
reduced dispersion of repolarization among the LV regions of an afflicted dog. In unafflicted dogs, isoproterenol did not change APD$_{90}$ significantly in any region (Figure 3B), whereas in afflicted dogs, it significantly shortened APD$_{90}$ anterobasally (where predrug repolarization was the longest), prolonged APD$_{90}$ anteroseptally (where predrug repolarization was the shortest), and had no significant effect postero-basally (compare Figure 3B with Figure 2B). As a result, isoproterenol minimized dispersion in different LV regions of afflicted dogs.

**Voltage Clamp Experiments**

Figure 4 shows the separation of $I_{Kr}$ and $I_{Ks}$ by the 2-tailed protocol. When the repolarization step from $+40$ mV was subdivided in a step to $0$ mV followed by a second step to $-40$ mV (Figure 4A), dofetilide had no significant effect on the tail at $0$ mV but almost completely inhibited the tail at $-40$ mV (Figure 4B), designating the latter as $I_{Ks}$. The dofetilide-resistant component (the tail at $0$ mV) was confirmed as $I_{Ks}$ by blockade with chromanol 293B (Figure 4C). Residual current after chromanol ranged from 0.03 to .11 pA/pF.

We used the 2-tailed protocol to compare $I_{Kr}$ and $I_{Ks}$ between anteroseptal and anterobasal regions in unafflicted and afflicted dogs since the maximum difference in APD$_{90}$ in afflicted dogs occurred between these regions (Figure 2). Control values for $I_{Ks}$ and $I_{Kr}$ did not differ between regions in unafflicted dogs (Figure 5, left). In afflicted animals, control $I_{Ks}$ was the same in both regions, but $I_{Kr}$ was smaller anterobasally than anteroseptally (Figure 5, right).

Isoproterenol increased $I_{Ks}$ but did not affect $I_{Kr}$ in either region of unafflicted dogs. Isoproterenol increased $I_{Ks}$ as well in both regions of afflicted animals. However, in contrast to unafflicted dogs, isoproterenol also appeared to increase $I_{Kr}$ (second tail amplitude) in the anterobasal region of afflicted animals. Because we could not be certain if the apparent effect of isoproterenol on $I_{Kr}$ was in fact exerted on this current or alternatively resulted from a shift in the $I_{Ks}$-voltage relation, we studied this further in anterobasal LV of unafflicted and afflicted dogs. In these experiments, cells were pretreated with $10^{-6}$ mol/L dofetilide to eliminate $I_{Kr}$. Control $I_{Ks}$ I-V curves did not differ between groups over the entire range of voltages (Figure 6, A and B). Isoproterenol increased $I_{Ks}$ significantly in cells from unafflicted dogs in the voltage range of $+30$ through $+70$ mV and in cells from afflicted dogs at voltages of $+30$ through $+50$ mV. The percent change in current induced by isoproterenol did not differ between groups.

Voltage-dependent activation curves for $I_{Ks}$ were obtained based on I-V relation data (Figure 6C). Isoproterenol produced a leftward shift in activation in both groups but more prominently in afflicted dogs (control-isoproterenol difference in $V_{1/2}=14.1\pm2.1$ mV, afflicted versus $8.5\pm1.1$ mV, unafflicted $P<0.05$). The greater shift induced by isoproterenol in cells from afflicted dogs can lead to a larger change in $I_{Ks}$ at threshold values near $0$ mV. This can account for the isoproterenol effect observed on the second tail in figure 5.

**Discussion**

Heterogeneity of repolarization may occur along and across the LV wall, with both contributing to T-wave morphology and arrhythmogenesis. Therefore, we analyzed both types of heterogeneity. We found that at low pacing rates...
there is significant spatial inhomogeneity of repolarization along the LV wall of afflicted but not unafflicted dogs, whereas transmural dispersion of repolarization does not differ between the 2 groups. Although 4000 ms represents a long cycle length, it is pathophysiological relevant, given the long pauses that have been recorded during sinus rhythm in afflicted animals. Such heterogeneity of repolarization can provide a substrate for reentry and pause-dependent ventricular tachycardia and might account for the abnormal T-wave morphology in afflicted dogs.

Our voltage-clamp data pinpoint the ionic basis of this heterogeneity. In afflicted animals, \( I_{\text{Kr}} \) was significantly larger and \( I_{\text{Ks}} \) significantly smaller anterobasally than anteroseptally whereas no differences in these currents occurred across the 2 LV regions in unafflicted dogs. For most action potentials we recorded, plateau potential was in the range of 0 to +10mV. At these potentials, \( I_{\text{Ks}} \) is fully activated, whereas \( I_{\text{Kr}} \) is relatively small. This indicates a greater dependence for repolarization on \( I_{\text{Ks}} \) and the smaller \( I_{\text{Kr}} \) can account for the long APD recorded anterobasally in afflicted dogs.

The occurrence of pause-dependent ventricular tachycardia at complete rest or in sleep is in a setting of low sympathetic tone. We therefore anticipated that any dispersion of repolarization in these animals should be maximal in the absence of catecholamine and should diminish in a setting of increased sympathetic tone. We simulated this using isoproterenol. Whereas isoproterenol had minor effects on repolarization in all regions of unafflicted dogs, it shortened APD anterobasally and prolonged it anteroseptally in afflicted animals, thereby equalizing LV repolarization. Thus, \( \beta \)-adrenergic stimulation would appear to eliminate the arrhythmogenic substrate for pause-dependent arrhythmias in afflicted dogs.

Our voltage-clamp data with isoproterenol explain these isolated tissue findings. The effects of isoproterenol were about the same in both LV regions of unafflicted dogs, increasing \( I_{\text{Ks}} \) and not changing \( I_{\text{Kr}} \). In afflicted animals, isoproterenol increased \( I_{\text{Ks}} \) similarly in both regions, whereas what appeared to be \( I_{\text{Kr}} \) in the 2-tailed protocol was significantly elevated anteroseptally. The latter result was somewhat unexpected because \( I_{\text{Kr}} \) is recognized to be regulated by \( \beta \)-adrenergic receptor stimulation, but controversy exists regarding the effects of \( \beta \)-adrenergic receptor activation on \( I_{\text{Kr}} \). No changes, stimulation or inhibition have been reported.
Because isoproterenol enhancement of what appeared to be \( I_{Kr} \) occurred only anterobasally, we compared isoproterenol effects on the voltage dependence of \( I_{Ks} \) activation in this region. The more prominent leftward shift of activation in afflicted than unafflicted animals significantly increased normalized \( I_{Ks} \) at 0 mV, thereby verifying that the isoproterenol-induced increase in what appeared to be \( I_{Kr} \) in the 2-tailed protocol was actually due to an enhanced contribution of \( I_{Ks} \) at this potential. The consequence of this \( I_{Ks} \) contribution to repolarization in the plateau voltage would be significant APD shortening by isoproterenol.

The heterogeneity in LV repolarization and isoproterenol effects derives most likely from a regional delay in the development of sympathetic innervation to the LV in afflicted dogs.\(^1\) There is considerable literature concerning the potential for delayed, incomplete, or asymmetric development of cardiac sympathetic nerves to be arrhythmogenic (see References 22 and 23). Moreover, sympathetic innervation is known to modulate the functional expression of repolarizing ionic currents in the developing heart.\(^2\) Reduced \( I_{Ks} \) density in LV regions having decreased innervation was demonstrated in afflicted German shepherds.\(^2\) Also favoring a role for sympathetic nerves as long-term modulators of the LV substrate are the observation that right-sided stellectomy to make regional sympathetic denervation at birth results in 4 to 5 months of altered LV electrophysiological properties similar to those observed in German shepherds afflicted with arrhythmias.\(^2\)

The present study strengthens the hypothesis that the mechanisms of pause-dependent and tachycardia-dependent lethal arrhythmias in afflicted German shepherds are different.\(^2\) Pause-dependent arrhythmias are most marked when the animals are lying quietly or are asleep.\(^2\) Ventricular tachycardia in these animals typically is initiated by a premature complex coupled to a sinus complex that follows a long pause. These arrhythmias are attributed to triggering by EADs originating in LV Purkinje fibers.\(^2\) The present study suggests a substrate for maintenance of these arrhythmias. In the absence of sympathetic stimulation and at a low heart rate, there is significant dispersion of repolarization in the LV of afflicted animals which may facilitate reentry.\(^2\) Increased sympathetic tone (\( \beta \)-adrenoreceptor stimulation) inhibits EADs in Purkinje fibers of afflicted animals and, as per the present study, equalizes LV repolarization, thus reducing both trigger and substrate for pause-dependent arrhythmias. Limiting the interpretation of our findings is that studies using MIBG to estimate extent and location of innervation, while implicating the regions to have studied here, were performed in different animals.\(^1\) Also, complicating the situation, isoproterenol-induced delayed afterdepolarizations (DAD) and DAD-induced triggered activity have recently been documented in LV sympathetic innervation in afflicted
dogs. These DADs may underlie catecholamine-sensitive and exercise-induced ventricular tachycardias. In conclusion, our data demonstrate significant spatial dispersion of repolarization in the LV of German shepherd dogs afflicted with arrhythmias. This finding is an important component relating the German shepherd model of lethal arrhythmias to human disease because heterogeneity of ventricular repolarization is also important in some inherited arrhythmias in human subjects. Moreover, as in afflicted dogs, abnormal sympathetic innervation has been identified in patients with Brugada syndrome and in some patients with congenital long QT syndrome.

With regard to the latter patient group, although ion channelopathies are recognized as determining the arrhythmogenic substrate (eg, Reference 31), the role of sympathetic nerves as a trigger remains an attractive hypothesis. Thus, the German shepherd model of inherited lethal arrhythmias mimics certain conditions (heterogeneity of ventricular sympathetic innervation and repolarization) important in humans, which makes its further investigation both important and promising.

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