Rate- and Site-Dependent Effects of Propafenone, Dofetilide, and the New $I_{Ks}$-Blocking Agent Chromanol 293b on Individual Muscle Layers of the Intact Canine Heart

Alexander Bauer, MD; Ruediger Becker, MD; Kirsten D. Freigang, MD; Julia C. Senges, MD; Frederik Voss, MD; Alexander Hansen, MD; Matthias Müller, MD; Hans Jochen Lang, PhD; Uwe Gerlach, PhD; Andreas Busch, PhD; Patricia Kraft, RN; Wolfgang Kübler, MD; Wolfgang Schölts, MD

Background—Recent in vitro studies have demonstrated regional differences in electrophysiological properties of individual left ventricular muscle layers. Controversy exists on the relevance of these findings for the situation in vivo. Thus, this study was designed to determine whether the in vivo canine heart exhibits regional differences in left ventricular refractoriness and in the susceptibility to sodium and potassium channel blockers.

Methods and Results—In 16 dogs, 36 needle electrodes (12 mm long, 4 bipolar electrodes, interelectrode distance 2.5 mm) were inserted into the left ventricular wall. By use of a computerized multiplexer-mapping system, the spread of activation in epicardial, endocardial, and midmyocardial muscle was reconstructed during ventricular pacing at 300- and 850-ms basic cycle length (BCL). Effective refractory periods (ERPs) were determined at baseline and after application of propafenone (2 mg/kg), dofetilide (30 μg/kg), or chromanol 293b (10 mg/kg) by the extrastimulus technique (BCL 300 and 850 ms). At baseline, activation patterns and ERPs were uniform in all muscle layers. Propafenone homogeneously decreased conduction velocity and moderately prolonged ERPs without any regional differences. Dofetilide and chromanol 293b did not affect the spread of activation. Dofetilide exhibited reverse use-dependent effects on ERP, still preserving transmural homogeneity of refractoriness. Chromanol 293b led to a regionally uniform but more pronounced increase in local ERPs at faster than at slower pacing rates.

Conclusions—At the heart rates applied, the in vivo canine heart does not exhibit regional differences in electrophysiological properties. Given the homogeneity of antiarrhythmic drug effects, induction of local gradients of refractoriness is obviously not a common mechanism of proarrhythmia in normal hearts. (Circulation. 1999;100:2184-2190.)

Key Words: antiarrhythmia agents ■ arrhythmia ■ electrophysiology ■ mapping

Recent studies suggest inhomogeneities in electrophysiological properties of individual muscle layers throughout the ventricular wall. Data were collected in isolated cells and in tissue preparations of ventricular myocardium.1-5 Sicouri and Antzelevitch5 were the first to describe a subpopulation of cells in subepicardial and midmyocardial layers of the canine ventricular wall in vitro. At very slow heart rates, these cells exhibited a dramatic increase in action potential duration. At faster rates, only slight transmural differences in repolarization were observed.4-6 Controversy exists as to whether regional differences in refractoriness are detectable in vivo, particularly in view of the relatively fast heart rate even after AV-node ablation. El Sherif and coworkers7 described marked dispersion of local refractoriness; others8,9 found only slight differences in repolarization throughout the canine left ventricle. Regional differences in refractoriness, if actually present, should be relevant, because local dispersion of refractoriness not only mediates QT prolongation and T/U wave abnormalities but also facilitates the induction of reentry and triggered activity.10-12 Dispersion of refractoriness might occur only after application of antiarrhythmic drugs. Regional differences in susceptibility to channel blockers could be based on regional differences in the expression of ion channels.6,11-14 Liu and Antzelevitch15 demonstrated the slowly activating component of the delayed rectifier potassium current to be less pronounced in midmyocardial and subepicardial cells than in epicardial and endocardial cells. Again, some in vitro data suggest differential effects of sodium channel blockers on individual muscle layers, which so far could not be confirmed in vivo.5,12,14

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Correspondence to Dr Alexander Bauer, Abteilung Innere Medizin III, Medizinische Universitätsklinik, Bergheimerstraße 58, 69115 Heidelberg, Germany. E-mail alexander_bauer@med.uni-heidelberg.de
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Thus, we applied 3D mapping techniques in dogs with acute AV block to determine activation and refractory patterns in individual muscle layers before and after application of propafenone, dofetilide, or chromanol 293b, respectively. Propafenone primarily affects sodium currents, and dofetilide and chromanol 293b the rapidly (Iₖ₉) and slowly (Iₖ₇) activating components of the delayed rectifier potassium current, respectively. The study was designed to determine whether the in vivo canine heart exhibits regional differences in left ventricular refractoriness and in the susceptibility to these antiarrhythmic drugs.

Methods

All animal experiments conformed to the “Position of the American Heart Association on Research Animal Use” adopted November 11, 1984. Studies were performed in 16 foxhounds weighing between 12 and 16 kg. Propafenone was applied in 6 dogs, and dofetilide and chromanol 293b in 5 dogs, respectively.

Model Preparation

Anesthesia was initiated and maintained with repeated boluses of pentobarbital (0.5 mg/kg IV). The dogs were intubated and ventilated with nitrous oxide and oxygen. Buprenorphin 0.3 mg IV was administered before any procedures were begun. ECG leads I, II, and aVF and aortic blood pressure were continuously monitored on a physiological recorder (VR12; Electronics for Medicine). Complete AV block was induced by transvenous radiofrequency catheter ablation (Cerablate plus 735 catheter, HAT 200 RF generator; Sulzer Osypka GmbH). The heart was exposed through a midsternotomy and suspended in a pericardial cradle. Thirty-six needle electrodes were inserted into the anterior left ventricular wall, organized in 6 rows and 6 columns parallel and perpendicular to the left anterior descending coronary artery (LAD), respectively (Figure 1), with an interneedle distance of ~10 mm. The chest was then covered, and body temperature was maintained at ~37°C with a heating lamp. During the experiment, demand pacing (60 bSource:JACC - June 2000

Needle Electrodes and Recording Instrumentation

The 36 custom-made needle electrodes were 12 mm long and 1 mm in diameter. Each needle contained 4 bipolar electrodes (interpolar distance 0.5 mm, interelectrode distance 2.5 mm). Thus, local electrograms were obtained from intramural sites 1, 4, 7, and 10 mm deep. All 144 electrograms were processed simultaneously through a 256-channel multiplexer and recorded and stored on videotape for offline digitization and computer analysis. Details of the multiplexer recording system and the methods for constructing isochronal activation maps have been reported previously.16-17 Electrograms were displayed, and a time window of interest was chosen. After digitization (sampling rate 1000 Hz), the moment of local activation for each electrogram was preselected by the computer, reviewed manually, and revised if necessary. In ECGs that showed a sharp intrinsic deflection, the maximum first derivative in slow multiphasic ECGs, the peak of the major deflection was taken as the moment of activation. Activation times were calculated relative to the pacemaker artifact. Based on local activation times, isochronal maps were constructed manually at 10-ms intervals.

Study Protocol

Measurements were performed at baseline and 15 minutes after intravenous application of 2 mg/kg propafenone (6 dogs), 30 µg/kg dofetilide (5 dogs), or 10 mg/kg chromanol 293b (5 dogs).

To determine the spread of activation in epicardial, endocardial, and midmyocardial muscle layers, ventricular pacing at 300- and 850-ms basic cycle lengths (BCLs) was applied through each bipole of the needle located next to the LAD and the base of the heart. Activation maps were constructed for each muscle layer. Total activation time was calculated as the difference between the earliest and the latest activation times recorded. In each dog, local effective refractory periods (ERPs) were determined at all 4 bipoles of 8:3 randomly selected needles. Thus, transmural resolution of ERP measurements was 3 mm. The distance between selected needles, however, could reach up to 30 mm. After 8 beats (S,) at 300- and 850-ms BCLs, the shortest S, coupling interval that depolarized the ventricle was defined as the local ERP. A maximum BCL of 850 ms was chosen to ensure overdrive suppression of the underlying escape rhythm. ERP measurements at 2 BCLs at each bipole before and after drug application already required 16 runs per needle. More BCLs or a larger number of S, stimuli would have prolonged measurements to such an extent that stable conditions could not have been maintained.

After the experiment, the heart was excised with the needles left in place. After fixation in formalin for at least 24 hours, the heart was cut transversely into slices ~8 to 10 mm thick. Insertion sites and directions of all pins were noted, and the position of each recording site was traced on a graphic representation of the left anterior wall, which served as matrix for reconstruction of the activation maps. Finally, the thickness of the left anterior wall was determined in each heart.

Statistical Analysis

Data are expressed as mean±SD. Student's t test for paired and unpaired data or ANOVA was applied where appropriate. A confidence level of 95% was considered statistically significant.

Results

With 16 experiments and 16 activation maps per experiment (4 muscle layers, 2 BCLs, control and drug values), 256 activation maps had to be constructed. Of the 36 electrograms per muscle layer, 92±4% proved to be of sufficient quality for a reliable analysis. Thus, >8400 electrograms were looked at in detail. Similarly, with 16 ERPs per needle in every experiment and 8±3 randomly selected needles per dog, >2000 refractory measurements were performed.

After AV-node ablation, none of the dogs developed significant ventricular tachyarrhythmias either at baseline or...
on drugs. Likewise, Vos et al. described a high incidence of arrhythmias, particularly of the torsade de pointes type, only in dogs with chronic but not with acute AV block.

Electrophysiological Properties of Individual Muscle Layers at Baseline

Baseline values were obtained in all 16 experiments. Activation maps revealed a homogeneous spread of activation from the site of stimulation across the left ventricular wall in all muscle layers. Regional conduction delay or block was not evident at any BCL. Slight directional differences in the spread of activation seemed to reflect fiber orientation in epicardial, endocardial, and midmyocardial muscle layers. A representative example is shown in Figure 2. Total activation times were similar for all muscle layers (Figure 3). Likewise, ERPs demonstrated physiological rate adaptation but no difference between individual muscle layers at either BCL (Figure 4).

Effects of Propafenone on Individual Muscle Layers

Propafenone seemed to slightly delay the spread of activation in all muscle layers. Accordingly, total activation times increased at both BCLs (Figure 5). Local ERPs were moderately prolonged without regional differences (Figure 6). At an 850-ms BCL, the increase in ERP at subepicardial sites (4 mm deep) did not reach statistical significance, although the changes in refractoriness were comparable to those elsewhere. Thus, this observation is probably not of biological significance. Except for subepicardial sites, the increase in ERP with propafenone tended to be more pronounced at slower than at faster pacing rates.

Effects of Dofetilide on Individual Muscle Layers

Dofetilide did not appear to affect the spread of activation in any muscle layer: activation maps remained virtually unchanged. Consequently, no systematic change in total activation times was detectable (Table 1). Local ERPs were homogeneously prolonged, again more markedly at slower than at faster pacing rates (Figure 7). Because of these uniform, reverse use-dependent effects of dofetilide, the existing transmural homogeneity in refractoriness was preserved, although at a different level.

Effects of Chromanol 293b on Individual Muscle Layers

As with dofetilide, the activation patterns did not reveal any significant changes after application of chromanol 293b, as substantiated by almost identical total activation times at baseline and on drug (Table 2). Chromanol 293b did, however, significantly prolong ERPs. Again, there was no evidence of a preferential effect on any muscle layer, so that the dispersion of refractoriness remained insignificant. Still, a striking difference between chromanol 293b and the other drugs emerged regarding the rate-dependence of the effects on refractoriness, which were significantly more pronounced at faster than at slower pacing rates, that is, positively use-dependent (Figure 8). At a BCL of 300 ms, local ERPs at some sites even exceeded the S1 S1 interval, thereby preventing 1:1 conduction. An increase in local pacing thresholds was excluded by demonstration of regular capture at longer basic intervals. For statistical analysis, local ERP at those sites was considered to be 300 ms.
Comparison of Early and Late ERP Measurements

Because ERP measurements with the extrastimulus technique are time-consuming, the stability of the preparation is a concern. If relevant changes in hemodynamics or temperature, for example, should occur, one would expect systematic differences in ERPs obtained early or late during the experiment. Thus, in each dog, ERPs determined at the first and last needle were compared and revealed no significant difference (Table 3).

Anatomic Findings

After excision and fixation of the heart, the positions of the needle electrodes relative to individual muscle layers could be verified. In >90% of all impalements, the very tip of the needles would perforate the endocardium, so that the most distal electrode pair was located subendocardially. The mean thickness of the left anterior wall was 9.2 ± 0.8 mm.

Discussion

Recent in vitro studies have highlighted differences in electrophysiological properties of isolated cell layers.1–4 Findings in vivo, however, were inconsistent. Anyukhovsky et al8,9 found no significant transmural dispersion of refractoriness, whereas El Sherif et al7 described heterogeneous repolarization intervals in epicardial, endocardial, and midmyocardial muscle layers. Like Anyukhovsky et al,8 we found homogeneous repolarization patterns throughout the intact left ventricular wall. There are, however, some methodological differences. Anyukhovsky et al8,9 relied on activation recovery intervals from bipolar recordings, which correlate with the duration of transmembrane action potentials but do not allow us to assess postrepolarization refractoriness.19 El Sherif and colleagues,7 using activation recovery intervals from unipolar recordings, which they could show to correlate with local ERPs in their model, suggested some regional inhomogeneity in repolarization at baseline. As pointed out by Anyukhovsky et al,9 intense stimulation of the cervical vagosympathetic trunks could have mediated this local dispersion of refractoriness. A preferential effect of antiarrhythmic drugs on individual cells or cell layers has again been indicated primarily by in vitro studies.1,5,7,12–14 This also relates to propafenone, a potent blocker of sodium channels with slight β-blocking and calcium-antagonistic effects.20 Effects on ERPs are generally moderate.21 Tamargo21 observed a significant depression of conduction throughout the left ventricle. Recovery of excitability in isolated muscle layers was found to be nonuniform, with a shortening of action potential duration in Purkinje fibers and a lengthening in ventricular muscle fibers. In a recent study with the IKr blocker E-4031, a preferential prolongation of the action potential duration was seen in M cells.22 Shimizu and Antzelevitch23 used chromanol 293b to mimic LQT1 circumstances in vitro. Only with β-adrenergic activation could they create transmural dispersion of repolarization in arterially perfused wedges of canine left ventricle exposed to IKr blockade, and only with β-adrenergic activation were they able to induce torsades de pointes.

Table 1. Total Activation Times at Baseline and After Application of Dofetilide During Pacing at BCLs of 300 and 850 ms

<table>
<thead>
<tr>
<th>Depth, mm</th>
<th>1</th>
<th>4</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 300</td>
<td>34±13</td>
<td>33±8</td>
<td>30±5</td>
<td>27±6</td>
</tr>
<tr>
<td>Dofetilide 300</td>
<td>29±5</td>
<td>31±7</td>
<td>33±5</td>
<td>27±6</td>
</tr>
<tr>
<td>Control 850</td>
<td>33±10</td>
<td>35±7</td>
<td>31±4</td>
<td>30±4</td>
</tr>
<tr>
<td>Dofetilide 850</td>
<td>29±6</td>
<td>34±5</td>
<td>31±6</td>
<td>29±7</td>
</tr>
</tbody>
</table>

Values are in ms, mean±SD.

Figure 5. Effects of propafenone on total activation times of individual muscle layers at different BCLs.

Figure 6. Effects of propafenone on local ERPs in individual muscle layers at different BCLs.

Figure 7. Effects of dofetilide on local ERPs in individual muscle layers at different BCLs.
TABLE 2. Total Activation Times at Baseline and After Application of Chromanol 293b During Pacing at BCLs of 300 and 850 ms

<table>
<thead>
<tr>
<th>Depth, mm</th>
<th>1</th>
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<th>7</th>
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<tbody>
<tr>
<td>Control 300</td>
<td>39±5</td>
<td>38±4</td>
<td>35±2</td>
<td>35±4</td>
</tr>
<tr>
<td>Chromanol 293b 300</td>
<td>36±6</td>
<td>36±5</td>
<td>34±3</td>
<td>32±5</td>
</tr>
<tr>
<td>Control 850</td>
<td>42±4</td>
<td>41±4</td>
<td>37±3</td>
<td>34±3</td>
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<tr>
<td>Chromanol 293b 850</td>
<td>35±4</td>
<td>38±5</td>
<td>37±6</td>
<td>37±2</td>
</tr>
</tbody>
</table>

Values are in ms, mean±SD.

TABLE 3. Early and Late ERP Measurements After Application of Propafenone, Dofetilide, or Chromanol 293b

<table>
<thead>
<tr>
<th>Depth, mm</th>
<th>1</th>
<th>4</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propafenone, early</td>
<td>243±16</td>
<td>249±12</td>
<td>238±20</td>
<td>238±18</td>
</tr>
<tr>
<td>Propafenone, late</td>
<td>244±24</td>
<td>256±16</td>
<td>246±19</td>
<td>245±18</td>
</tr>
<tr>
<td>Dofetilide, early</td>
<td>243±23</td>
<td>246±38</td>
<td>241±33</td>
<td>246±37</td>
</tr>
<tr>
<td>Dofetilide, late</td>
<td>256±35</td>
<td>256±38</td>
<td>257±36</td>
<td>264±38</td>
</tr>
<tr>
<td>Chromanol 293b, early</td>
<td>298±42</td>
<td>289±32</td>
<td>273±14</td>
<td>287±18</td>
</tr>
<tr>
<td>Chromanol 293b, late</td>
<td>286±21</td>
<td>288±21</td>
<td>300±41</td>
<td>292±19</td>
</tr>
</tbody>
</table>

Values are in ms, mean±SD.

Provided that transmural dispersion of repolarization is crucial for the occurrence of torsade de pointes, our findings would support that concept: With the conditions applied, transmural dispersion of refractoriness was not evident, and consequently, no tachyarrhythmias were observed.

Liu and Antzelevitch demonstrated a weaker current density of the delayed rectifier in M cells than in myocytes from the epicardium or endocardium. These regional differences in the expression of channel proteins could modulate the susceptibility of individual cells to electropharmacological interventions. Our findings, however, are not in line with this hypothesis. Propafenone, dofetilide, and chromanol 293b uniformly prolonged ERPs in all muscle layers, and their rate-dependent behavior was also independent of the site of action. Likewise, Anyukhovsky et al found the type I agent quinidine to homogeneously affect the canine ventricular myocardium in vivo.

The reasons for the apparent differences between in vitro and in vivo preparations are not clear. Obviously, the isolation of cells or cell layers could be relevant. Cell-to-cell interactions within the intact structure of the in situ heart probably synchronize the repolarization process. Furthermore, individual muscle layers in the working heart are exposed to different degrees of wall stress. Wall stress might modulate electrophysiological properties and thus compensate for existing regional differences, which become unmasked with the loss of the normal contractile function in vitro. Similar considerations might apply to neurohumoral effects. Finally, the pacing rates applied in vitro were much slower than those in vivo. If functional differences between individual cells are detectable only under extreme conditions, however, their physiological significance seems questionable.

Reasons for the apparent lack of regional differences in the susceptibility to antiarrhythmic drugs in vivo are also speculative. Local concentrations of respective agents might play a role. With very high doses of a drug, blockade of certain channels might be ubiquitously complete, so that regional differences in channel expression are of minor relevance. Furthermore, drug effects can be modified by changes in heart rate, autonomic tone, or hemodynamics, all factors being distinctively different in in vitro and in vivo preparations.

The rate dependence seen with propafenone, dofetilide, and chromanol 293b was consistent within the various muscle layers. Chromanol 293b exhibited positive use-dependent effects on refractoriness, that is, more marked prolongation with increasing pacing rates. This could be a result of its slow kinetics but also of accumulation of the I\(_K_s\) current with faster heart rates, by which it becomes the major component of the delayed rectifier. At present, we are not aware of any study describing the rate-dependent effects of chromanol 293b in vivo. At least in theory, positive use-dependence seems to be an ideal property of an antiarrhythmic drug, because it would exert its action primarily when it is needed the most, that is, at fast heart rates. Likewise, prolongation of action potential duration would be minimal at slow heart rates, which would reduce the risk for proarrhythmia.

Methodological Considerations

The insertion of multiple plunge electrodes might cause significant injury and change electrophysiological properties. However, Pogwizd and Corr compared epicardial activation maps derived from contact electrodes and from plunge electrodes, which did not reveal a significant difference. Likewise, our activation maps provided no evidence for marked conduction delay or conduction block.

As in comparable studies, we chose the maximum first derivative of the local electrogram to determine the moment of local activation. Because activation maps reflect the sequence of activation at multiple recording sites relative to each other, the criterion applied to determine local activation
is of minor relevance as long as all electrograms are analyzed identically.

The extrastimulus technique does not allow us to assess dynamic changes in ERP. Furthermore, it is time-consuming and requires stable conditions for a long time. The concordance of early and late measurements suggests that this was actually the case.

With 8 S1 stimuli, steady-state conditions were probably not reached. However, the emphasis of our study was on the comparison of individual muscle layers. Because the methodological limitations apply to epicardial, endocardial, and midmyocardial measurements alike, their relation should not have been significantly affected.

Recordings 10 mm from the epicardium might not be sufficiently deep to visualize the endocardium. However, the thickness of the left anterior wall in the relatively small dogs used in our study was 9.2 ±0.8 mm on average, and the location of the needle electrodes was verified after the experiments.

In 34 healthy mongrel dogs weighing 8 to 12 kg, Kamimura and colleagues26 found the thickness of the left anterior wall to average 7.1 ± 1.2 mm.

Recently, Yan et al27 showed a more subendocardial location of the so-called M-cell layer in the intact left anterior wall of the canine ventricle. Thus, the issue of accurately determining ERPs in the subendocardium becomes more relevant. With the most endocardial electrode of our needles recording from a depth of ~10 mm and a mean thickness of the left anterior wall of 9.2 ± 0.8 mm, the endocardium should have been adequately covered. ERPs could be determined at all endocardial sites, even though in some cases ≤ 1 pole of the electrode was already located within the ventricular cavity. The second most endocardial electrode reached a depth of 7 mm. Thus, a relatively thin (≤3 mm) specific muscle layer could have been missed because of the resolution of the ERP measurements.

To allow for measurements at slow heart rates, the AV node was ablated. Comparable studies have also used AV blockade8-9 or intense vagal stimulation.7

Still, the functional and electrophysiological consequences of acute AV block could have interfered with preexisting regional differences in activation and/or refractoriness. The marked increase in cardiac volume is only partly accounted for by the increase in contractile performance. Electrophysiologically, there is increased wall stress, and there could also be an effect of cardiac memory. However, one would have to speculate that such electrophysiological effects do have a regional preference and that the relative change in refractoriness always just compensates for existing regional differences.

Clinical Implications

The use of antiarrhythmic drugs is limited by their potential for proarrhythmia. Local dispersion of refractoriness, either preexisting or in response to electropharmacological interventions, is considered to be of major relevance for arrhythmogenesis. Although this seems to be true for diseased myocardium, our data suggest that the physiological properties of the normal heart do not facilitate this mechanism.

Acknowledgments

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