Potassium Channel Subunit Remodeling in Rabbits Exposed to Long-Term Bradycardia or Tachycardia

Discrete Arrhythmogenic Consequences Related to Differential Delayed-Rectifier Changes

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Background—Sustained heart rate abnormalities produce electrical remodeling and susceptibility to arrhythmia. Uncontrolled tachycardia produces heart failure and ventricular tachyarrhythmia susceptibility, whereas bradycardia promotes spontaneous torsade de pointes (TdP). This study compared arrhythmic phenotypes and molecular electrophysiological remodeling produced by tachycardia versus bradycardia in rabbits.

Methods and Results—We evaluated mRNA and protein expression of subunits underlying rapid (I\textsubscript{Kr}) and slow (I\textsubscript{Ks}) delayed-rectifier and transient-outward K\textsuperscript{+} currents in ventricular tissues from sinus rhythm control rabbits and rabbits with AV block submitted to 3-week ventricular pacing either at 60 to 90 bpm (bradypaced) or at 350 to 370 bpm (tachypaced). QT intervals at matched ventricular pacing rates were longer in bradypaced than tachypaced rabbits (eg, by 50% at 60 bpm; P<0.01). KvLQT1 and minK mRNA and protein levels were downregulated in both bradypaced and tachypaced rabbits, whereas ERG was significantly downregulated in bradypaced rabbits only. Kv4.3 and Kv1.4 were downregulated by tachypacing only. Patch-clamp experiments showed that I\textsubscript{Kr} was reduced in both but I\textsubscript{Ks} was decreased in bradypaced rabbits only. Continuous monitoring revealed spontaneous TdP in 75% of bradypaced but only isolated ventricular ectopy in tachypaced rabbits. Administration of dofetilide (0.02 mg/kg) to mimic I\textsubscript{Kr} downregulation produced ultimately lethal TdP in all tachypaced rabbits.

Conclusions—Sustained tachycardia and bradycardia downregulate I\textsubscript{Kr} subunits, but bradycardia also suppresses ERG/I\textsubscript{Ko}, causing prominent repolarization delays and spontaneous TdP. Susceptibility of tachycardia/heart failure rabbits to malignant tachyarrhythmias is induced by exposure to I\textsubscript{Kr} blockers. These results point to a crucial role for delayed-rectifier subunit remodeling in TdP susceptibility associated with rate-related cardiac remodeling. (Circulation. 2006;113:345-355.)

Key Words: arrhythmia • electrophysiology • ion channels • long-QT syndrome • torsade de pointes

Sustained heart rate abnormalities produce ventricular electrical remodeling and susceptibility to cardiac arrhythmia. Uncontrolled tachycardia produces heart failure and ventricular tachyarrhythmia susceptibility, whereas bradycardia promotes spontaneous torsade de pointes (TdP). Here, we directly compare the consequences of sustained tachycardia and bradycardia on rabbit QT intervals and arrhythmic phenotypes, and evaluate subunit remodeling changes in animal models of sustained tachycardia and bradycardia, and the spontaneous arrhythmic syndromes associated with such models have not been directly compared.

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Repolarization delays caused by ionic remodeling are common in hypertrophy and HF. Reduced transient-outward current (I\textsubscript{o}) is common in HF. Transcriptional downregulation of Kv4.3 subunits underlies I\textsubscript{Kr} reduction in human HF. In chronic AVB-induced remodeling, I\textsubscript{oa} is unchanged, but decreased rapid (I\textsubscript{Kr}) and slow (I\textsubscript{Ks}) delayed-rectifier K\textsuperscript{+} currents are seen. Reduced I\textsubscript{Kr} appears to be due to transcriptional downregulation of the α-subunit KvLQT1 and the β-subunit minK in canine AVB. Limited information is available on other K\textsuperscript{+} channel subunit expression changes in animal models of sustained tachycardia and bradycardia, and the spontaneous arrhythmic syndromes associated with such models have not been directly compared.

Here, we directly compare the consequences of sustained tachycardia and bradycardia on rabbit QT intervals and spontaneous arrhythmias and relate these to mRNA and membrane-protein expression changes in subunits underlying I\textsubscript{Ko}, I\textsubscript{Kr}, and I\textsubscript{Ks}. Because of discrepancies in previous findings, we also evaluate functional changes in I\textsubscript{Ko} and I\textsubscript{Kr}.

Methods

Animal handling followed the Guide for the Care and Use of Laboratory Animals (NIH publication 85–23); procedures were...
approved by the Animal-Experimentation Ethics Committee of the Montreal Heart Institute.

**Experimental Protocol**

New Zealand white rabbits (2.0 to 3.0 kg) were used. We created AVB and implanted programmable right ventricular (RV) pacemakers (Medtronic) as previously described. AVB was created in all 44 bradycardia rabbits and in 32 tachycardia rabbits (to monitor their rhythm at heart rates similar to those of bradycardia rabbits). Fifteen additional tachypaced rabbits without AVB were studied to assess ECG interval changes. After AVB creation, ventricular pacing (180 bpm, near-physiological rabbit heart rate) was performed for 5 days. A day 0 baseline ECG was then recorded under ketamine (25 mg/kg)/xylazine (5 mg/kg) anesthesia. In tachycardia rabbits, tachypacing was then programmed (350 bpm for 1 week, then 370 bpm for 2 weeks), as previously described. Bradycardia rabbits were subjected to VVI pacing (90 bpm for 1 week, followed by 60 bpm for 2 weeks), as previously described. Bradycardia rabbits had no signs of HF but biventricular HF, including biventricular dilation, ascites, and pleural effusions. Tachycardia rabbits had signs of HF, including biventricular dilation, ascites, and pleural effusions. Bradycardia rabbits had no signs of HF but biventricular hypertrophy.

At the end of bradypacing or tachypacing periods, rabbits were euthanized (pentobarbital, 40 mg/kg IV). RV and left ventricular (LV) free walls were removed. LV samples were divided midway (LV) free walls were removed. LV samples were divided midway.

**ECG Recordings**

Once weekly (days 0, 7, 14, and 21), ECGs were obtained under ketamine/xylazine anesthesia at pacing rates of 180 and 60 bpm and then recorded continuously for 30 minutes at pacing rates of 60 to 90 bpm to observe spontaneous ventricular arrhythmias. ECG parameter measurements were based on averages of 3 consecutive beats in the lead providing the clearest QT-interval definition. QT dispersion (QTd) was defined as the time between peak and end of T waves.

A total of 44 bradycardia and 32 tachycardia rabbits entered the protocol. The detection function in Medtronic Kappa was set to detect and store ventricular high-rate episodes (>400 bpm) in 24 bradycardia rabbits and in 10 tachycardia rabbits with pacemakers reprogrammed to 90 bpm at ∼day 21. In addition, 24-hour ECG monitoring was performed by implanted telemetry in 2 bradycardia and 7 tachycardia rabbits. Ventricular tachyarrhythmias were defined as ≥8 consecutive beats at >400 bpm.

**Competitive RT-PCR**

Total RNA was isolated from 0.5- to 1.0-g samples with TRIzol reagent (Invitrogen), followed by chloroform extraction and isopropanol precipitation. Genomic DNA was eliminated by incubation in DNase I (0.1 U/μL, 37°C) for 30 minutes, followed by acid phenol-chloroform extraction. RNA was quantified by spectrophotometric absorbency (260 nm); purity was confirmed by the A260/A280 ratio; and integrity was evaluated by ethidium bromide staining on a denaturing agarose gel. RNA mimics were synthesized as previously described, including 460-bp rabbit α-actin sequences flanked by gene-specific primers and an 8-nucleotide linker homologous to the 3′-end of T7 promoter. Primer sequences and PCR conditions are provided in Table 1. Serial mimic dilutions were mixed with 1-μg RNA samples in 20-μL reaction mixtures for RT. RT products were used as templates for subsequent PCR with gene-specific primers. PCR products were visualized under ultraviolet light with ethidium bromide, captured with a Nighthawk camera, and quantified with Quantity-One software (PDI). A DNA mass marker (100 ng) was used to determine size and quantity of DNA bands and to create standard curves for absolute quantification. Logarithmic plots [LN([target]/[mimic]) versus LN([mimic])] were fit by linear regression to quantify mRNA.

**Western Blots**

Membrane fractions were prepared as previously described. Protein samples were separated with SDS PAGE, transferred to Immobilon-P polyvinylidene fluoride membranes, and blocked in Tris-buffered saline/5% nonfat milk. Primary antibodies were incubated for 18 hours at 4°C (minK) or 1 to 2 hours at room temperature (Kr ERG, Kv4.3, and Kv1.4). Antibodies to KvLQT1 (sc-10646; goat), minK (sc-16796; goat), ERG-1a (sc-15968; goat), and Kv4.3 (sc-11686; goat) were purchased from Santa Cruz Biotechnology; and anti-Kv1.4 (APC007; rabbit) was purchased from Alomone. Monoclonal KChIP2 antibody was kindly supplied by James Trimmer. Secondary antibodies were horseradish peroxidase–conjugated donkey anti-goat IgG and anti-rabbit IgG (Santa Cruz).

Band signals were detected and quantified with Western-Lightning Chemiluminescence Reagent Plus, laser scanning, and Quantity-One software.

**Patch-Clamp Experiments**

LV cardiomyocytes were isolated as described previously. Sham-operated sinus rhythm rabbits were used as controls. All rabbits for cell isolation were euthanized at ∼day 21. Membrane currents were recorded by tight-seal whole-cell patch clamp with Na⁺-free, K⁺-free extracellular solutions including N-methyl-d-glucamine to eliminate inward-rectifier K⁺ current (I_K). Cell capacitance was determined with ramp pulses (0.5 V/s) from −50 to 70 mV. Cell capacitance and series resistance (R_s) were compensated by ~50% to 70%. I_ks was recorded in the presence of the highly selective I_ks blocker HMR-

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**TABLE 1. Primers for RT-PCR**

<table>
<thead>
<tr>
<th>Current</th>
<th>Clone</th>
<th>Primer Pair</th>
<th>Position, bp</th>
<th>Size, bp</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_K</td>
<td>KvLQT1 F: GCCGCAGCAAAGTTAGTCTG R: CCTTCTTAGCCAGTACAGA</td>
<td>86–402</td>
<td>317</td>
<td>57.6</td>
<td></td>
</tr>
<tr>
<td>minK</td>
<td>F: CCGTATGCCCCTTTGACC R: GTACCCTGTTCCTTTCTCTG</td>
<td>23–285</td>
<td>263</td>
<td>58.4</td>
<td></td>
</tr>
<tr>
<td>I_K</td>
<td>ERG F: TGGACCATTTAGCAAGATC R: GGATGACGACCTCCACAC</td>
<td>936–1198</td>
<td>263</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>I_K</td>
<td>Kv4.3 F: CCACCTGGTGTCTCCTATCT R: TCCGTCCTGCTCTTCA</td>
<td>1457–1800</td>
<td>344</td>
<td>55.4</td>
<td></td>
</tr>
<tr>
<td>I_K</td>
<td>Kv1.4 F: TATGCCGTATGGTTATGC R: TGGTGGACTCGCTTCTT</td>
<td>17–259</td>
<td>243</td>
<td>56.8</td>
<td></td>
</tr>
</tbody>
</table>

F indicates forward; R, reverse.
Data Analysis

All data are expressed as mean±SEM. Each biochemical determination was performed on an individual rabbit. Western blot band intensities are expressed after background subtraction as optical density units (ODUs) normalized to GAPDH signal intensity for the same sample. There were no significant differences between LV endocardial and epicardial results, so data from each rabbit were averaged and presented as a single LV value. For the ECG parameters, 2-way repeated-measures ANOVA was performed using mixed model methodology with group and time as main effects. In case of a significant interaction between the 2 main effects, Dunnett contrasts were used to compare them over time within each group. The mixed procedure and multiple comparison with Dunnett’s test were used to evaluate differences in mRNA and protein expression among groups over time. Statistical comparisons for ionic current data were obtained by ANOVA, followed by Tukey’s test. Values of P<0.05 indicated statistical significance. All probability values in the figures are based on ANOVA, followed by Dunnett’s or Tukey’s test.

Results

ECG Changes

Figure 1 shows ECGs at matched pacing rates in a bradycardic and a tachypaced rabbit. Day 21 QT intervals were prolonged in the bradycardic rabbit but were unchanged in the tachypaced rabbit. Figure 1C summarizes ECG changes. QRS duration did not change. In bradycardia rabbits, QT intervals increased from 256±3 ms at 60 bpm (day 0) to nearly steady-state values (332±9 ms) by day 7. In tachycardia rabbits, QT intervals decreased from 256±5 ms at day 0 to 223±5 ms at day 7. During sinus rhythm recording (available only for tachypaced rabbits not subjected to AVB), corrected QT intervals increased from 145±3 ms (day 0) to 158±2 ms (day 21; P<0.05). QTd increased only in bradycardia rabbits.

Spontaneous Ventricular Arrhythmias

Of 44 rabbits entering the bradycardia group, 23 died before day 21. Sixty-one percent (14 of 23) died suddenly, with ECG recordings showing spontaneous TdP. The remaining 9 died...
of unknown causes. The bradypaced rabbits surviving for 21 days had $66\pm17$ (range, 2 to 232) episodes per rabbit of TdP. Thirteen and 5 bradypaced rabbits were euthanized for molecular studies and patch-clamp experiments, respectively, at $\sim$day 21. Spontaneous TdP was documented in 33 of 44 bradypaced rabbits (75%). Figure 2A shows intracardiac electrograms stored by implanted pacemakers in 2 bradypaced rabbits. Rabbit 1 developed polymorphic VT at day 7 and died the next day. Rabbit 2 had frequent TdP-like VT before death at day 16. Figure 2B shows continuous telemetry from a bradypaced rabbit. Five days after pacing was set to 60 bpm, spontaneous TdP occurred and degenerated to VF. All rabbits were subjected to a 30-minute ECG recording on day 21. Of 21 bradypaced rabbits surviving to day 21, 16 (76%) showed spontaneous TdP during this recording.

Of the 32 tachypaced rabbits, 21 survived for 21 days, and 11 died prematurely. No tachyarhythmias were detected with the episode report function of implanted pacemakers. Continuous 24-hour telemetry ECG recording of 7 tachycardia rabbits after pacing rate reduction to 60 to 90 bpm showed a single 7-complex run in 1 (Figure 2C), isolated ventricular premature beats in 3, and no arrhythmia in 3. Of 21 tachypaced rabbits monitored by ECG for 30 minutes on day 21, none showed spontaneous VT (versus 76% of bradypaced rabbits; $P<0.001$).

**Expression of $I_{KS}$ Subunits**

Examples of competitive RT-PCR gels for KvLQT1 and minK are shown in Figure 3A. Lane 0 contains 100 ng of DNA mass ladder to create the standard curve. Lanes 1 through 6 were obtained with serial dilutions of the RNA mimic, along with 1 µg total RNA per lane. Top bands represent RNA mimic products. Bottom bands are target bands coamplified with mimics in the same reaction tube. As mimic concentration decreases from left to right, the target band becomes stronger, demonstrating mimic-target competition. Overall, mRNA expression was significantly reduced for both $I_{KS}$ subunits in bradypaced and tachypaced rabbits (Figure 3A, bottom). In bradypaced rabbits, KvLQT1 expression was reduced by averages of 76% and 53% (RV and LV, respectively) and minK by 58% and 43%. In tachypaced rabbits, KvLQT1 expression was reduced by 64% and 51% (RV and LV) and minK by 74% and 43%.

Figure 3B shows KvLQT1 and minK Western blots on 1 gel each. A prominent KvLQT1 band was observed at the expected molecular mass of $\sim$75 kDa. Antibody preincuba-
tion with antigenic peptide eliminated the signal. The minK blot showed a faint signal at the expected molecular mass of 27 kDa, consistent with previous reports of human, guinea pig, and rabbit minK signals at 27 kDa.15 This signal disappeared on preincubation with antigenic peptide, unlike the higher-molecular-mass signals. KvLQT1 protein expression was reduced by 72% and 53% in the RV and LV, respectively, among bradypaced rabbits and by 78% and 53% for tachypaced rabbits. MinK was downregulated similarly in tachypaced (by 45% and 37% in the RV and LV) and bradypaced (by 37% and 33%) rabbits.

**ERG Expression**

A typical ERG competitive RT-PCR gel for a control rabbit is shown in Figure 4A. ERG mRNA was significantly downregulated in bradypaced rabbits only (Figure 4B; expression in bradycardia rabbits reduced by 59% in the RV and 52% in the LV). Representative ERG Western blots are shown in Figure 4C. A clear band at the expected molecular mass of 110 kDa was suppressed in bradypaced rabbits, and lower-molecular-weight bands sometimes appeared (3 of 7). All signals were eliminated by antibody preincubation with antigenic peptide. ERG protein expression was significantly downregulated in bradypaced rabbits only (Figure 4D; by 50% in the RV and 37% in the LV).

**Expression of I\textsubscript{\textit{to}} Subunits**

Figure 5A shows representative competitive RT-PCR gels for Kv4.3 and Kv1.4, as well as mean data. Kv4.3 and Kv1.4 mRNA expression was downregulated in tachypaced rabbits (Kv4.3: by 53% in RV, 26% LV; Kv1.4: by 77% in RV, 59% LV). Kv4.3 mRNA was unchanged in bradypaced rabbits, whereas Kv1.4 mRNA showed a small decrease of borderline statistical significance in the RV and no significant change in the LV. Kv4.3 antibody revealed bands at 78 and 70 kDa (Figure 5B), consistent with long and short splice variants in rabbit ventricle.17 For Kv1.4, 2 clear bands were detected close to the expected molecular mass of 97 kDa. I\textsubscript{\textit{to}} subunit protein expression was significantly reduced in tachypaced rabbits only, by 60% and 74% (Kv4.3 and Kv1.4, respectively) in the RV and 43% and 36% (Kv4.3, Kv1.4) in the LV. KChIP2 expression was comparable in control, tachypaced, and bradypaced rabbits (eg, averages in the LV were 3.4±0.8, 3.1±1.1, and 3.2±0.6 ODUs, respectively).
Changes in $I_{Kr}$ and $I_{Ks}$

Previous data on delayed-rectifier current downregulation in tachypacing-induced HF have varied. To correlate directly delayed-rectifier subunit expression and current alterations in our rabbits, we performed patch-clamp experiments. Typical $I_{Kr}$ tail currents were seen in cells from all groups but were smaller in bradypaced rabbits (Figure 6A). $I_{Ks}$ was smaller in both bradypaced and tachypaced rabbits (Figure 6B). Figure 7 shows activation voltage dependence (top) and tail current-voltage relations (bottom). $I_{Kr}$ (Figure 7A) activated at more negative voltages than $I_{Ks}$ (Figure 7B), following well-recognized properties. $I_{Kr}$ activation voltage dependence was not altered in either bradypaced (V1/2, $-7.0\pm 2.5$ mV; control, $-5.5\pm 1.9$ mV) or tachypaced (V1/2, $-8.7\pm 2.0$ mV; control, $-8.7\pm 1.7$ mV) rabbits. $I_{Ks}$ activation voltage dependence was similarly unaffected (V1/2, $9.3\pm 4.4$ mV for bradypaced, $17.8\pm 3.9$ mV for tachypaced, 14.7±1.7 mV for control; P=NS). $I_{Ks}$ tail current density was reduced significantly in bradypaced rabbits (by $\approx 40\%$) but was unchanged in tachypaced rabbits (Figure 7C). $I_{Ks}$ tail current density was reduced by $\approx 70\%$ and $\approx 55\%$ in bradypaced and tachypaced rabbits, respectively (Figure 7D). There were no differences among groups in $I_{Kr}$ and $I_{Ks}$ deactivation kinetics (Table 2).

Response of Tachypaced Rabbits to $I_{Kr}$ Inhibition

Our arrhythmia-monitoring data showed that bradypaced but not tachypaced rabbits were highly susceptible to spontaneous TdP. $I_{Ks}$ and its subunits KvLQT1 and minK were
downregulated similarly by bradypacing and tachypacing, but only bradypacing suppressed ERG/IKr. We hypothesized that IKr reduction might be crucial for differential TdP susceptibility in bradypaced rabbits. We therefore tested the effect of selective IKr blockade with a single intravenous dose of dofetilide (0.02 mg/kg), followed by telemetric monitoring for 3 hours during pacing at ~90 bpm in tachypaced rabbits, to mimic IKr downregulation in bradypaced rabbits. The dose of dofetilide was based on previous studies showing that normal rabbits receiving the same dose per minute (total dose 60 times as large) developed TdP and VF with incidences of 2.5% and 17%, respectively. Figure 8A shows continuous monitoring after dofetilide administration to a tachypaced rabbit. The QT interval was markedly prolonged, and TdP occurred frequently. All 6 tachypaced rabbits given dofetilide displayed TdP that degenerated into VF. Spontaneous ventricular tachyarrhythmia prevalence in bradypaced rabbits, tachypaced rabbits without dofetilide exposure, and tachypaced rabbits treated with dofetilide is summarized in Figure 8B. Dofetilide increased VT occurrence in tachypaced rabbits.
from 0% to 100% \( (P<0.0001, \text{ Fisher's exact test} \) to a level comparable to bradypaced rabbits (75%). QT intervals and QTd at 600-ms cycle length were much smaller in tachypaced than bradypaced rabbits in the absence of dofetilide (Figure 8C); however, dofetilide exposure increased values in tachypaced rabbits to levels comparable to those in bradypaced animals.

### Discussion

In this study, we compared directly the K\(^+\) channel subunit remodeling and arrhythmic phenotypes resulting from persistent bradycardia versus tachycardia in rabbits. Whereas bradypaced rabbits displayed downregulation of both rapid and slow components of the delayed-rectifier current system, only slow-component subunits and current were suppressed in tachypaced rabbits. This differential ion channel regulation was associated with differential susceptibility to spontaneous ventricular tachyarrhythmias, with bradypaced rabbits showing frequent TdP and tachypaced rabbits manifesting limited spontaneous ventricular arrhythmia. The arrhythmic response of tachypaced rabbits to dofetilide, which transformed their arrhythmic phenotype toward that of bradycardia rabbits, supports the notion that the particular TdP diathesis of bradycardic rabbits is due to \( I_{Kr} \) downregulation.

### Relationship to Previous In Vivo Studies of Rate-Related Remodeling

The tachypacing model of HF is well established for studies of electrophysiological remodeling.\(^2,8–10,14,17,18,20,21\) \( I_{Kr} \) downregulation is the most consistent finding. The response of delayed-rectifier currents has been more variable; although decreases in \( I_{Ks} \) have been observed consistently,\(^14,18,21\) some studies reported decreased \( I_{Ks} \)\(^14\) and others indicated no change.\(^18,21\) In bradycardia-related remodeling, \( I_{Ks} \) is consistently downregulated.\(^6,22\) \( I_{Ks} \) has been reported to be decreased in bradycardic rabbits\(^6\) and decreased in RVs but not LVs of bradycardic dogs.\(^22\) In this study, \( I_{Ks} \) was reduced in both bradycardic and tachycardic rabbits, but \( I_{Kr} \) was reduced only

### Table 2. Deactivation Time Constants on Repolarization From 50 to −50 mV

<table>
<thead>
<tr>
<th></th>
<th>Control, ms</th>
<th>Bradypaced, ms</th>
<th>Tachypaced, ms</th>
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<tbody>
<tr>
<td>( I_{Kr} )</td>
<td>112±21 (6)</td>
<td>771±131 (6)</td>
<td>92±14 (8)</td>
</tr>
<tr>
<td>( I_{Ks} )</td>
<td>92±8 (5)</td>
<td>357±96 (5)</td>
<td>111±25 (5)</td>
</tr>
</tbody>
</table>

Values in parentheses are numbers.
in bradycardia, consistent with changes in mRNA and protein expression of corresponding subunits. In a previous study,\textsuperscript{14} $I_{Kr}$ was reportedly downregulated in tachycardic rabbits; however, 50% activation voltages for $I_{Kr}$ and $I_{Ks}$ were similar, suggesting possible contamination of E-4031 sensitive currents by $I_{Ks}$ rundown.

The propensity of bradycardia-remodeled hearts to TdP is well documented.\textsuperscript{3–6} Pak et al. observed nonsustained VT and 1 recorded lethal ventricular tachyarrhythmia in 25 dogs with tachypacing-induced HF. A propensity to early afterdepolarization-related arrhythmias can be unmasked in tachypaced HF by repolarization-prolonging interventions,\textsuperscript{2,23} suggesting decreased repolarization reserve consistent with the reductions in $I_{Kr}$ and associated subunits observed in the present study.

There has been limited work on remodeling of ion channel subunits in animal models involving sustained heart rate abnormalities. Ramakers et al\textsuperscript{13} observed decreases in both KvLQT1 and minK expression in bradycardic dogs. We were unable to identify other studies of $K^+$ channel subunit remodeling in bradycardic animal models. In tachycardia remodeling, downregulation of Kv4.x subunits is consistently observed.\textsuperscript{17,20,24} Akar et al\textsuperscript{24} found a tendency to reduced ERG mRNA in tachypaced dog hearts, although a band of unspecified molecular weight was increased on Western blot. No changes in $I_{Kr}$ subunits were seen. Similarly, Rose et al\textsuperscript{20} did not find significant changes in delayed-rectifier $K^+$ channel subunits in tachypaced rabbits despite significantly decreased delayed-rectifier currents ($I_{Kr}$ and $I_{Ks}$ were not separated electrophysiologically). In addition to decreased ERG mRNA, our results point to possible posttranslational changes in bradypaced rabbits, because in some cases we observed lower-molecular-weight bands possibly corresponding to immature ERG protein.

**Novel Findings and Potential Significance**

The present study is the first to compare directly arrhythmic phenotypes and ion channel remodeling in bradycardic versus tachycardic animal models. We have observed substantial differences in spontaneous ventricular arrhythmias, with TdP being ubiquitous in bradycardia-dependent remodeling and never occurring in tachypaced animals. This discrepancy appears to be due to differences in ERG/I_{Kr} remodeling. Evidence for the importance of bradycardia-specific ERG remodeling included reduced $I_{Kr}$ and ERG mRNA and protein, as well as the transformation of the ECG and arrhythmic phenotype of tachypaced rabbits toward that of bradypaced rabbits with $I_{Ks}$ inhibition.

These experimental phenotypes are likely relevant to several clinical phenomena. AVB has been recognized since the 1920s\textsuperscript{25} as a precipitant of TdP, and TdP is a recognized presentation of congenital heart block.\textsuperscript{26} Patients with AVB and spontaneous TdP require pacing to at least 70 bpm to prevent excess QT prolongation.\textsuperscript{27} It has long been thought that the promotion of TdP by bradycardia is due to the acute heart rate–APD relationship, but our studies suggest an intriguing additional possibility: downregulation of ion channel subunits (and in particular ERG) by bradycardia. This notion may also account for another potentially important phenomenon: the ability of chronic $\beta$-blocker therapy to increase APD. Unexplained APD-prolonging effects of chronic (but not acute) in vivo $\beta$-blockade have been observed in animal\textsuperscript{28} and human\textsuperscript{29} studies, as well as in isolated human atrial myocytes.\textsuperscript{30} Such effects are potentially relevant to mechanisms of $\beta$-blocker antiarrhythmic actions and to the varying efficacy of $\beta$-blockade in patients with congenital long-QT syndrome.

We did not observe spontaneous TdP in tachypaced rabbits with HF; however, TdP occurred in all rabbits on dofetilide exposure. These observations may parallel the uncommon occurrence of TdP in HF patients, despite a clearly increased risk of TdP in HF patients exposed to $I_{Kr}$-blocking drugs.\textsuperscript{31} These findings are consistent with the concept of “repolarization reserve.”\textsuperscript{32} Downregulation of $I_{Kr}$ subunit expression and function may not in itself be sufficient to cause spontaneous TdP, but in the setting of superimposed $I_{Ks}$ inhibition or downregulation, repolarization may become unstable and lead to early afterdepolarizations and related tachyarrhythmias.

**Potential Limitations**

We cannot exclude the possibility that the abnormal activation sequence caused by ventricular pacing contributed to ion channel remodeling. However, if dyssynchrony were a prime factor, we would have expected to see differential remodeling of LV endocardium, epicardium, and RV, which was not the case. It is unlikely that AVB per se contributed to ion channel remodeling, because KvLQT1 protein expression was reduced to the same extent in tachycardia rabbits without (0.12±0.03 ODU) and with (0.10±0.02 ODU) AVB compared with controls (0.36±0.09 ODU).

We were surprised to see QT shortening in tachypaced rabbits with AVB (Figure 1C). This may have resulted because the pacing rate (180 bpm) during the 5-day recovery interval after AVB, before tachypacing, was slower than the spontaneous sinus rate (∼250 bpm), possibly causing APD prolongation at day 0. This notion is supported by the fact that QT intervals during sinus rhythm increased slightly but significantly over time in tachypaced rabbits without AVB (Figure 1C). The small QT interval increase in the face of significant downregulation of KvLQT/minK and Kv4.3/Kv1.4 subunits encoding $I_{Kr}$ and $I_{Ks}$ may be due to several factors. $I_{Ks}$ is small in the rabbit because of low-level minK expression,\textsuperscript{15} and pure $I_{Ks}$ suppression fails to increase APD in the rabbit.\textsuperscript{31} In addition, because of the very slow recovery kinetics of rabbit $I_{Ks}$, it contributes very little current at physiological heart rates.\textsuperscript{34}

Our minK protein bands were very weak, consistent with known low-level expression in the rabbit,\textsuperscript{15} but limiting the reliability of the measurement and requiring caution in interpretation. Therefore, conclusions about the protein expression of this subunit need to be particularly guarded. We elected to perform patch-clamp studies only for delayed-rectifier currents because of previous discrepancies in the literature. Because $I_{Kr}$ has been consistently found to be downregulated in HF,\textsuperscript{2,9,14,18,20,21} a result consistent with our molecular determinations, we did not prepare additional rabbits for $I_{Kr}$ measurement and therefore lack such results for direct correlation with biochemical data.
The response of tachypaced rabbits to dofetilide supports the notion that \( I_K \) downregulation is the major factor determining arrhythmic phenotype differences between brady-paced and tachypaced animals. We studied a limited number of ion channel subunits and currents. The potential roles of other ion channels and transporters, structural remodeling, differences in autonomic or neurohumoral response, altered activation sequence, mechanical factors, etc, require further exploration. In addition, we did not explore the issue of whether tachypaced rabbits were more susceptible to dofetilide-induced TdP than control rabbits. A full exploration of the potential electrophysiological and arrhythmic consequences of reduced repolarization reserve in brady-paced and tachypaced rabbits would have required extensive additional experiments and, while interesting and important, is beyond the scope of the present paper.

We were unable to separate a distinct midmyocardial layer because of the thinness of the rabbit ventricle. We did evaluate separately ion channel expression changes in subepicardial versus subendocardial tissues and did not observe any significant differences.

The arrhythmic phenotype we noted in tachypaced HF rabbits was different from that reported previously by Pogwizd et al in rabbits with HF after aortic banding/regurgitation. The differences are likely due to the different methods of HF induction but emphasize the importance of not generalizing excessively from a single animal model of HF. Differences in underlying cause, duration, and severity of HF likely differentially influence the resulting electrophysiological remodeling and arrhythmic phenotype of HF in humans.

**Acknowledgments**

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**Disclosures**

None.

**References**


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**CLINICAL PERSPECTIVE**

Chronic tachycardias produce HF associated with sudden cardiac death. Persistent bradycardia can cause acquired long-QT syndrome and the polymorphic VT TdP. Potassium currents, including the rapidly activating and slowly activating delayed-rectifier currents, are important determinants of repolarization and the QT interval. The present study tested the idea that cardiac potassium channels are remodeled by sustained heart rate abnormalities and that this remodeling predisposes to arrhythmias. Rabbits subjected to AV block and persistent bradypacing developed marked QT prolongation and recurrent TdP. Bradycardia reduced mRNA and protein expression of both rapid and slow delayed-rectifier potassium channel subunits and their corresponding ionic currents in ventricular myocytes. In contrast, rabbits subjected to sustained rapid ventricular pacing developed heart failure but minimal spontaneous ventricular arrhythmias, and only the slow component of the delayed rectifier potassium current was reduced. Administration of dofetilide to block the rapid delayed-rectifier to tachycardia-remodeled rabbits produced marked QT-interval prolongation and TdP. These results show that sustained abnormalities in heart rate remodel cardiac ion channel expression. Decreases in both components of the delayed-rectifier current, causing TdP, can occur with chronic bradycardia alone or with tachycardia-induced heart failure, combined with administration of an agent that blocks the rapid delayed-rectifier current. This remodeling likely contributes to the predisposition of patients with prolonged bradycardia to TdP and may explain previously noted repolarization changes with chronic β-blocker therapy. It may also contribute to sudden death in patients with heart failure, particularly in the presence of sustained tachycardia, by sensitizing the heart to factors that impede cardiac repolarization.
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