Atrial Fibrillation in KCNE1-Null Mice

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Abstract—Although atrial fibrillation is the most common serious cardiac arrhythmia, the fundamental molecular pathways remain undefined. Mutations in KCNQ1, one component of a sympathetically activated cardiac potassium channel complex, cause familial atrial fibrillation, although the mechanisms in vivo are unknown. We show here that mice with deletion of the KCNQ1 protein partner KCNE1 have spontaneous episodes of atrial fibrillation despite normal atrial size and structure. Isoproterenol abolishes these abnormalities, but vagomimetic interventions have no effect. Whereas loss of KCNE1 function prolongs ventricular action potentials in humans, KCNE1−/− mice displayed unexpectedly shortened atrial action potentials, and multiple potential mechanisms were identified: (1) K+ currents (total and those sensitive to the KCNQ1 blocker chromanol 293B) were significantly increased in atrial cells from KCNE1−/− mice compared with controls, and (2) when CHO cells expressing KCNQ1 and KCNE1 were pulsed very rapidly (at rates comparable to the normal mouse heart and to human atrial fibrillation), the sigmoidicity of Ik, activation prevented current accumulation, whereas cells expressing KCNQ1 alone displayed marked current accumulation at these very rapid rates. Thus, KCNE1 deletion in mice unexpectedly leads to increased outward current in atrial myocytes, shortens atrial action potentials, and enhances susceptibility to atrial fibrillation. (Circ Res. 2005;97:62-69.)

Key Words: arrhythmia ■ atrial fibrillation ■ cardiac electrophysiology ■ mouse ■ potassium channels

Atrial fibrillation (AF) is a major public health problem, affecting 2% of people over the age of 60 and 9% of people over 80.1 AF is the proximate cause of 20% to 25% of strokes and is implicated in disability attributable to heart failure.2 Despite the magnitude of this problem, the development of increasingly sophisticated animal models, and advances in pharmacological and nonpharmacological therapy, our understanding of the molecular effector mechanisms underlying AF remains incomplete.3

Whereas the critical mass hypothesis suggested that the atrial action potential is governed by the amount of KCNQ1 expressed,4,5 spontaneous atrial arrhythmias are now well-documented in mice with atrial dilation.6–9 However, atrial dilation results in multiple changes in gene expression that complicate identification and validation of AF effector molecules. A strong familial component in AF10–12 suggests that identification of predisposing DNA variants may provide new insights into underlying biology. Indeed, rare kindreds with a high incidence of AF have been described, and 3 disease genes, each linked to arrhythmias in a single kindred,13–15 as well as 3 other loci,16–18 have been reported. Mutations in one of these disease genes, KCNQ1, also cause the most common form of the congenital long QT syndrome.19 KCNQ1 associates with the ancillary subunit KCNE1 to generate the adrenergically regulated20 potassium current IKs. Mutations in either gene reduce IKs, prolong action potentials in human ventricle and thereby generate the long QT phenotype. Interestingly, AF-associated mutations in either KCNQ1 or the KCNE2 subunit both generated gain-of-function defects in the expressed K+ current,13,15 and the predicted action potential shortening is well-recognized as predisposing to AF.3

We report here that mice in which KCNE1 has been knocked out display frequent spontaneous episodes of AF reversible by isoproterenol, and have structurally normal hearts. Further, we demonstrate shortened atrial action potentials and identify conditions under which KCNE1 deletion leads to action potential shortening. Taken together, these studies implicate KCNE1 as a modulator of the AF phenotype in this murine model.

Materials and Methods

Genotyping

The KCNE1 knockout/LacZ knockin mice were generated on a C57BL/6Nbackground, as previously described.21 Offspring of heterozygous matings were genotyped by PCR, and 3- to 6-month-
old wild-type (WT) (+/+) and knockout KCNE1−/− littermates were used in this study.

Ambulatory ECGs
Thirty-minute recordings were obtained in conscious unrestrained mice after intraperitoneal placement of telemeters (DSI) under ketamine and pentobarbital (0.033 mg/gm intraperitoneally [IP]) each. Leads were tunneled subcutaneously from the abdomen to the right and left shoulders (lead 1). Mice recovered for >24 hours before baseline recordings and provocative testing.

Analysis of Ambulatory Recordings
Rhythm in ambulatory unrestrained mice was analyzed by plotting all telemetered RR intervals during 30 minutes of recording, followed by inspection and classification by the following methods. Raw electrocardiographic data were reanalyzed using custom software for high accuracy QRS complex detection. Direct inspection with parallel analysis of raw and QRS-triggered signal-averaged segments was used to facilitate recognition of low-magnitude P wave signals. Plots of successive RR intervals were inspected for abrupt changes to exclude annotation artifacts and enhance episode detection. AF was defined as a rhythm during which P waves were absent and RR intervals became irregular (Figure 1).

Shorter-term measures of heart rate variability depend on mean heart rate; to analyze changes in this relationship, mean RR interval and measures of variability were computed in each individual 3-second epoch in the 30-minute recordings, excluding all episodes of AF. Baseline 30-minute data segments were obtained on 2 to 3 separate days in each animal. All analyses were conducted by an investigator blinded to genotype.

Drug Challenge
Ambulatory ECGs were recorded for 30 minutes before any intervention. Dose-response curves for change in heart rate 5 minutes after intraperitoneal challenge with carbachol, phenylephrine, or isoproterenol (each 0.1 to 30 μg/kg) were determined. Succeeding doses were administered only after heart rate had returned to previous baseline.

Transesophageal Pacing
Under ketamine and pentobarbital anesthesia, transesophageal pacing was accomplished using a 2 French quadripolar catheter with 2-mm interelectrode spacing (Arrow International) inserted orally to a depth of 3 to 4 cm. Capture at <1.5 mA at 1.5 ms was obtained using minimal adjustments in the insertion depth. Body temperature was maintained using a warming light, and ECGs were recorded during atrial burst pacing using a commercially available software and hardware system (Gould Instrument Systems).

Transtracheal Echocardiography
Under ketamine/pentobarbital anesthesia, views for measurement of chamber size were obtained with a 15-MHz Hewlett Packard transducer.22 Left atrial size was measured in the long axis parasternal view in which the aorta, mitral valve, and LA were simultaneously visualized, and shortening fraction was measured in the short axis parasternal view at the level of the papillary muscles.

Electrophysiological mapping of the sinus node region was accomplished at 37.5±0.3°C using high-resistance K+-filled electrodes.23 Electrical maps were aligned with β-galactosidase staining by backfilling an electrode with 1% Alcian blue in 0.5 mmol/L sodium acetate (pH 4.0) at the end of the mapping procedure. The tip of the electrode was broken to lower resistance, and the tissue was marked with 10- to 50-μm-diameter Alcian blue dots iontophotographically. One dot was placed at the site of the dominant pacemaker, and 4 dots were placed at the corners of the nodal area. After fixation and washing, the preparations were permeabilized at room temperature and stained with permeabilization solution plus 1 mg/mL X-Gal, 5 mmol/L potassium ferricyanide, and 5 mmol/L potassium ferrocyanide in the dark at room temperature. After washing in PBS, preparations were fixed for a further hour and stored in 70% ethanol until use. β-galactosidase-stained preparations were dehydrated through 50%, 70%, 95%, and 100% ethanol into 100% isopropanol and paraffin embedded.

Standard sections (H and E and Masson trichrome staining) were reviewed under light microscopy by a cardiovascular pathologist (I.B.A.) blinded to the mouse genotype.

Mouse Atrial Myocyte Isolation and Recording
Myocyte isolation methods24 are presented in the online data supplement, available at http://circres.ahajournals.org. Standard methods, identical to those recently reported,25 were used to record potassium currents in atrial myocytes (at room temperature) and to record \( I_{Ks} \) and \( I_{KCNE1} \) in transfected CHO cells (37°C).
Analysis of KCNE Transcripts
Real-time RT-PCR (TaqMan) was performed on an ABI PRISM 7700 (Applied Biosystems) to quantify the changes in mRNA expression levels of KCNE subunits in WT and KCNE1−/− mice. Total RNA was isolated from the hearts of WT and KCNE1−/− littermates (n=5 pairs) using the acid/phenol method. The RNA was treated with DNase I to ensure the complete removal of genomic DNA and repurified using a Qiagen RNeasy Mini Kit. The integrity of the RNA was verified on a formaldehyde-containing gel. Total RNA was reverse transcribed into cDNA using random hexamer primers and the TaqMan Reverse Transcription Reagents Kit. Gene-specific primer sets and TaqMan MGB probes (see supplemental Table I) were selected using the Primer Express Software v2.0 and designed to span exon–exon boundaries to select against amplification of contaminating genomic DNA. Oligos were synthesized by MWG Biotech, and all probes carried the FAM label against amplification of contaminating genomic DNA. Oligos were amplified by the gene-specific primers were subcloned into the Topo TA Cloning Vector (Invitrogen) and then used as templates for the gene-specific primers to construct a standard curve. The concentrations of the plasmids used to generate the standard curve were measured using the PicoGreen dsDNA Quantitation Reagent (Molecular Probes). All real-time PCR reactions were performed in triplicate, and the experimental samples were processed on the same 96-well plate with the standards. The C\textsubscript{t} values of the experimental samples were normalized against those of an endogenous mouse \( \beta \)-actin control.

Data Analysis
Data in WT and KCNE1−/− animals were compared using Student t test. The incidence of pauses and AF at baseline and induced with transesophageal pacing was compared using Fisher exact test. All tests were 2-tailed, with a P value <0.05 considered significant. Data are presented as mean±1 SD.

Results
AF in KCNE1−/− Mice
Episodes of spontaneous AF were identified in 6 of 6 KCNE1−/− mice and in 0 of 5 WT littermates (Figure 1). AF was present in 14 of 16 30-minute recordings in KCNE1−/− animals versus 0 of 15 WT littermates. Mean AF episode duration was 6.4±9.3 sec; the mice were in AF 86±198 sec per 30 minutes recording (4.8% of the time; range 0 to 684 sec). The majority showed no major antecedent change in heart rate as in Figure 1; however, 13 of 214 episodes were preceded by heart rate slowing or atrioventricular block (Figure 2a); the bottom tracing in Figure 2a shows a rhythm seen on one occasion that most resembles atrial flutter. KCNE1−/− mice also displayed enhanced susceptibility to AF with atrial burst pacing, which provoked AF in 7 of 13 KCNE1−/− mice versus 1 of 13 WT (\( P=0.045 \); Figure 2b).

Mechanisms of AF Susceptibility: Whole Heart and Tissue Studies
Supplemental Table II demonstrates that weight, ventricular fractional shortening, and left atrial size were no different in KCNE1−/− and WT animals. Light microscopy revealed no enhanced fibrosis, myocardial disorganization, or other pathology in either group. These findings argue against a primary role for chamber dilatation in AF. When the mice were in sinus rhythm, there was no difference in RR intervals (102±5 [WT] versus 105±10) or in its variability, assessed as SD\textsubscript{RR} (3.2±1.3 versus 3.7±2.0; supplemental Table II). To further analyze heart rate variabil-
Mechanisms of AF Susceptibility: In Vitro Studies

Figure 4 and supplemental Table III show that action potentials from atrial free wall cells were significantly shorter in KCNE1−/− mice (APD90: 74.6 ± 15.2 ms [WT] versus 41.4 ± 11.5 ms; P<0.05), and this would be entirely consistent with enhanced AF susceptibility exhibited both as spontaneous episodes as well as episodes with burst pacing.3 However, such action potential shortening does not immediately follow from what has previously been understood to be the major role of KCNE1, enhancement of Ik,A amplitude and slowing of its activation.27 Were the situation as simple as this, deletion of KCNE1 would reduce Ik,A and increase action potential duration, and indeed exactly this action has been invoked in KCNE1-linked long QT syndrome.28 Accordingly, further experiments were conducted to determine mechanisms underlying this action potential shortening. Figure 6 shows that potassium currents recorded from KCNE1−/− atrial myocytes were significantly larger than those recorded from WT littermates (Figure 6). In addition, the component of outward current sensitive to the KCNQ1 blocker chromanol 293B29 was significantly larger in the knockout myocytes. Recent studies have indicated that chromanol 293B is not entirely specific for KCNQ-mediated channels and can block transient outward (Ito) and ultra-rapidly activating currents (Ikur) in human and dog atrial cells.30,31 To assess the possibility that KCNE1 deletion altered expression of other K+ channel subunits, we conducted real-time PCR experiments. Figure 7 (top) shows that the abundance of mRNA encoding the potassium channel genes thought to underlie Ik,A (KCN5A) and Ito (KCND2 and KCND3) were unaltered in the KCNE1−/− mice. Thus the chromanol 293B data support the idea that the increase in K+ current was attributable to increased KCNQ1-mediated current. KCNQ1 interacts not only with KCNE1 (to generate Ik,A) but also with other members of the KCNE family.32–35 Figure 7 (bottom) shows that the abundance of transcripts encoding other KCNE family members was not changed in the KCNE1−/− animals, whereas those for KCNE1 were absent, as expected.

Figure 8 shows a second possible explanation whereby KCNE1 deletion could lead to apparently paradoxical action potential. Ik,A displays sigmoidal activation kinetics after depolarizing pulses to plateau voltages.36 Figure 8 shows that at very fast stimulation rates (10 Hz), this distinctive feature of Ik,A gating prevents accumulation of activating current. By contrast, current generated by KCNQ1 alone displays no delay in activation, and thus accumulates even at extraordinarily fast rates. Hence, whereas the absence of KCNE1 confers a prolonged action potential phenotype at usual...
human heart rates, this effect is absent at very fast heart rates, similar to those seen in the mouse heart and in human AF, where accumulation of \( I_{KCNQ1} \) dominates, with enhanced outward current and shortening action potentials.

**Discussion**

We demonstrate here that KCNE1 disruption produces paroxysmal AF in the absence of complicating structural abnormalities. Further, we have identified shortening of atrial action potentials—well-known to generate an AF-prone substrate—as an important electrophysiologic consequence of KCNE1 disruption, and have suggested two mechanisms both involving enhanced outward current that may underlie this effect in the mouse heart. Isoproterenol abolishes paroxysmal AF in the KCNE1\(^{-/-}\) animals, although other indices of sympathetic input, including studies of sinus rate itself, show only subtle abnormalities in heart rate variability. The mechanism underlying modulation of AF by sympathetic activation in this model remains to be explored.

We suggest that shortened atrial action potentials provide the substrate for AF in this model, as in other settings. The trigger is presumably a sinus beat or an atrial ectopic engaging the AF-prone substrate; although it is possible that...
KCNE1 deletion promotes ectopic atrial activity to initiate AF, isolated PACs were not observed in the analyses conducted as in Figure 1. Outward current in dissociated atrial myocytes is increased in the KCNE1-null animals. An obvious question then is which outward current is enhanced, and experiments were therefore conducted with the $I_{KCNQ1}/I_K$ blocker chromanol 293B. The data in Figure 6 show clearly that an essential component of the increase is chromanol 293B-sensitive; this increase is seen during the depolarizing pulses, as well as the tails after repolarization. Inhibition of current by chromanol 293B reflects an interaction of the drug with the KCNQ1 subunit, and thus drug-sensitive current in the knockout may reflect either KCNQ1 channels alone, or those associated with other members of the KCNE family, such as KCNE2, KCNE3, and KCNE4. Prominent effects of such KCNQ1–KCNE interactions include altered current amplitude and altered gating; the absence of KCNE1 may thus enhance current mediated by KCNE subunits (whose mRNA is unchanged; Figure 7) to shorten action potentials. We find no change in KCNQ1 protein abundance in KCNE1−/− mice, also supporting the idea that reduced or altered partnering of the channel with remaining subunits explains the shortened action potentials we observe here. This line of reasoning is consistent with “gain-of-function” mutations reported in KCNQ1 or KCNE2 in familial AF, although the in vivo function of these mutants has not yet been described, the reported in vitro effect to increase outward current predicts shortening of atrial action potentials.

The data shown in Figure 8 indicate that the electrophysiologic consequences of KCNE1 deletion will be critically dependent on underlying rate, as previously suggested by studies of QT in anesthetized KCNE1−/− mice. In CHO cells driven at very rapid rates, the sigmoidicity displayed by activating $I_K$ (generated by multimerization of KCNQ1 and KCNE1) results in much less current accumulation compared with $I_{KCNQ1}$ alone. This experiment provides a second potential explanation for how KCNE1 deletion could increase outward current, shorten atrial action potentials, and thus predispose to AF. This formulation would apply not only to murine arrhythmias but also to the fibrillating human atrium, where shortened atrial action potentials would play a role in arrhythmia perpetuation. Another interesting prediction from these data are that the clinical phenotype of patients with LQT5 (loss of function KCNE1 mutations, in whom KCNQ1-mediated currents may still be present) may differ from that in patients with LQT1 (loss of function KCNQ1 mutations).

In Figure 2a, is that isoproterenol abolishes pauses and thereby the arrhythmia. Arguing against this as the sole mechanism is the fact that most AF episodes we observed did not display this pause-dependence. Activation of protein kinase A by adrenergic stimulation in mammalian heart has multiple downstream effects, and so the salutary effect of isoproterenol likely has multiple underlying mechanisms that remain to be fully defined. Other effects of adrenergic stimulation in the human heart include increased L-type calcium current and increased heart rate. Whereas the predicted result of these effects is increased ventricular action potential duration, and its surface correlate the QT interval, adrenergic stimulation of $I_K$ serves to limit this QT effect. Indeed, absence of increased $I_K$ with adrenergic stimulation likely underlies the propensity of patients with LQT1 to develop arrhythmias with physical or emotional stress. By contrast, the murine heart rate is so fast and ventricular repolarization so highly dependent on the transient outward current, that it is difficult to demonstrate altered ventricular repolarization with KCNE1 deletion. We find no change whereas others describe abnormal QT dependence on heart rate at very slow rates induced by anesthesia. Nevertheless, the findings here, obtained in the absence of confounding effects of anesthesia and by action potential recordings in vitro, convincingly demonstrate a role for KCNE1 is generating an AF phenotype in mice, a species not prone to this arrhythmia.

**Limitations**

A mechanism whereby KCNE1 deletion shortens atrial action potentials has not been definitively established here. We offer 2 possibilities, increased outward current (Figure 6) or a switch from $I_K$ to $I_{KCNQ1}$ (Figure 8), and these are not mutually exclusive. However, if the switch to $I_{KCNQ1}$ were the exclusive mechanism, we would expect a greater increase in outward current early during depolarizing pulses, which was not seen. Moreover, not all the difference in currents shown in Figure...
6 can be attributed to a chromanol 293B-sensitive component; indeed, even if it were, chromanol 293B is not selective for KCNQ1 channels but may also affect other currents, as discussed above.\textsuperscript{30,31} We have previously reported that KCNE1 expression is confined to the conduction system,\textsuperscript{21} although even in that study, atrial staining was presented. Finally, differences between murine and human repolarization could limit the extent to which the present data informs human arrhythmia mechanisms.

The identification of AF in a mouse with a single disrupted gene is an important step in efforts to identify molecular mechanisms and pathways whose dysfunction leads to this very common clinical problem, and adds to 2 kindreds in this potassium channel family are associated with AF.\textsuperscript{13,15} In addition, a small association study has reported over-representation of a common nonsynonymous mutation in this potassium channel family that play key roles in AF,\textsuperscript{13} and thus point to new methods of identifying and treating patients at risk.

Acknowledgments

This work was supported by National Institutes of Health grants (HL46681, HL49989). D.M.R. is the holder of the William Stokes chair in Experimental Therapeutics, a gift of the Dai-ichi Corporation.

References


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_Circ Res._ 2005;97:62-69; originally published online June 9, 2005;
doi: 10.1161/01.RES.0000173047.42236.88

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Mouse atrial myocyte isolation: Mouse hearts were removed after methoxyflurane anesthesia and cervical dislocation. The atria were then dissected away from the remaining supraventricular structures in Tyrode's solution containing (in mM) 140 NaCl, 5.4 KCl, 1 MgCl₂, 1.8 CaCl₂, 5 HEPES, and 5.5 D-glucose, warmed to 35°C; pH was adjusted to 7.4 by the addition of NaOH. The atria were then cut into strips and transferred to a solution containing (in mM) 140 NaCl, 5.4 KCl, 0.07 CaCl₂, 0.5 MgCl₂, 1.2 KH₂PO₄, 50 taurine, 5.5 D-glucose, 1 mg/ml bovine serum albumin (BSA), and 5 HEPES; pH was adjusted to 7.4. After three washes in this low Ca²⁺-containing solution, tissue strips were digested by the addition of collagenase type II (227 U/ml, Worthington Biochemical; Lakewood NJ), elastase (2.4 U/ml, Worthington), and protease type XIV (0.35 U/ml, Sigma; Oakville, Ontario, Canada). Enzymatic digestion took place for 30–35 min at 35°C, with manual agitation every 5 min. Tissue strips were then washed five times in a modified Kraftbrühe solution containing (in mM): 100 K⁺ glutamate, 10 K⁺ aspartate, 25 KCl, 10 KH₂PO₄, 2 MgSO₄, 20 taurine, 5 creatine base, 0.5 EGTA, 5 HEPES, 20 D-glucose, and 1 mg/ml BSA (pH adjusted to 7.2 with KOH). After this washing procedure, single atrial myocytes were isolated by gentle trituration at room temperature with the use of a fire-polished Pasteur pipette (3 mm inner diameter). Aliquots of this cell suspension were monitored by using a phase-contrast microscope (Zeiss ID 03) as trituration progressed. Trituration was continued until an acceptable yield of single atrial myocytes was achieved, usually within 10 min. The myocytes were then readapted to normal extracellular Ca²⁺ concentrations by the addition of solution containing 10 mM NaCl and 1.8 mM CaCl₂, followed by normal Tyrode's solution containing 1 mg/ml BSA. Cells were then stored in a solution containing (in mM) 100
NaCl, 35 KCl, 1.3 CaCl$_2$, 0.7 MgCl$_2$, 14 K$^+$ glutamate, 2 KH$_2$PO$_4$, 2 taurine, and 1 mg/ml BSA, until used in electrophysiology experiments.
### On-line table 1: KCNE Real-time PCR primer sets

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<th>TaqMan Probe</th>
<th>Amplicon size (basepairs)</th>
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### On-line Table 2: *In vivo* properties of *KCNE1<sup>−−</sup>* animals

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<th>Wild type</th>
<th><em>KCNE1&lt;sup&gt;−−&lt;/sup&gt;</em></th>
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<tr>
<td>RR during sinus rhythm (ms)</td>
<td>102±10</td>
<td>105±8</td>
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<tr>
<td>SD&lt;sub&gt;RR&lt;/sub&gt;</td>
<td>3.2±1.3</td>
<td>3.7±2.0</td>
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<td>RR during isoproterenol (ms)</td>
<td>86±6</td>
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<td>Weight (gm)</td>
<td>24±3.0</td>
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<td>ventricular fractional shortening (%)</td>
<td>54±9</td>
<td>55±7</td>
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<tr>
<td>left atrial size (mm)</td>
<td>1.28±0.13</td>
<td>1.45±0.10</td>
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</tbody>
</table>

RR: interbeat interval; SD<sub>RR</sub>: standard deviation of the RR interval. Isoproterenol data obtained 5 minutes after 1 µg/kg intraperitoneally.
On-line Table 3: *In vitro* sino-atrial node electrophysiology properties of *KCNE1*⁻/⁻ animals

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<td>(N=4, n=19)</td>
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<td>141±4</td>
<td>149±15</td>
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<td>33.7±9.9</td>
<td>17.6±6.0*</td>
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<td>Atrial APD&lt;sub&gt;90&lt;/sub&gt; (ms)</td>
<td>74.6±15.2</td>
<td>41.4±11.5*</td>
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<td>Sinoatrial conduction time (ms)</td>
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<th>Secondary pacemaker (N=3, n=10)</th>
<th>Primary pacemaker (N=3, n=7)</th>
<th>Secondary pacemaker (N=3, n=7)</th>
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<td>−54±8</td>
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<td>APA (mV)</td>
<td>56±2</td>
<td>55±6</td>
<td>54±3</td>
<td>60±5</td>
</tr>
<tr>
<td>APD&lt;sub&gt;50&lt;/sub&gt; (ms)</td>
<td>31±1</td>
<td>31±2</td>
<td>34±1</td>
<td>35±1</td>
</tr>
<tr>
<td>APD&lt;sub&gt;100&lt;/sub&gt; (ms)</td>
<td>82±1</td>
<td>80±5</td>
<td>73±6</td>
<td>78±6</td>
</tr>
<tr>
<td>DDR (mV/s)</td>
<td>117±20</td>
<td>70±28</td>
<td>134±22</td>
<td>60±32</td>
</tr>
<tr>
<td>dV/dt&lt;sub&gt;max&lt;/sub&gt; (V/s)</td>
<td>6.2±2</td>
<td>27±14</td>
<td>6.7±2</td>
<td>17±12</td>
</tr>
</tbody>
</table>

RR: interbeat interval; MDP: maximal diastolic potential; APA: action potential amplitude; APD<sub>50</sub>, APD<sub>90</sub>, and APD<sub>100</sub>: action potential duration at 50, 90 and 100% repolarization (respectively); DDR: diastolic depolarization rate during the first 20 ms after maximum diastolic potential; dV/dt<sub>max</sub>: maximum upstroke velocity. N: number of animals; n: number of cells. *P<0.05 vs wt.

Primary (dominant) and secondary (latent) pacemaker cells were readily distinguished: primary pacemakers had a diastolic depolarization rate > 85 mV/s and a maximal upstroke velocity < 10 Volts/sec.
Reference List
