Ionic Mechanisms of Regional Action Potential Heterogeneity in the Canine Right Atrium

Jianlin Feng, Lixia Yue, Zhiguo Wang, Stanley Nattel

Abstract—Atrial action potential heterogeneity is a major determinant of atrial reentrant arrhythmias, but the underlying ionic mechanisms are poorly understood. To evaluate the basis of spatial heterogeneity in canine right atrial repolarization, we isolated cells from 4 regions: the crista terminalis (CT), appendage (APG), atrioventricular ring (AVR) area, and pectinate muscles. Systematic action potential (AP) differences were noted: CT cells had a “spike-and-dome” morphology and the longest AP duration (APD; value to 95% repolarization at 1 Hz, 270±10 ms [mean±SEM]); APG and pectinate muscle cells had intermediate APDs (180±3 and 190±3 ms, respectively; P<0.001 versus CT for each), with APG cells having a small phase 1; and AVR cells had the shortest APD (160±4 ms, P<0.001 versus other regions). The inward rectifier and the slow and ultrarapid delayed rectifier currents were similar in all regions. The transient outward K+ current was significantly smaller in APG cells, explaining their small phase 1 and high plateau. L-type Ca2+ current was greatest in CT cells and least in AVR cells, contributing to their longer and shorter APD, respectively. The E-4031–sensitive rapid delayed rectifier K+ current was larger in AVR cells compared with other regions. Voltage- and time-dependent current properties were constant across regions. We conclude that myocytes from different right atrial regions of the dog show systematic variations in AP properties and ionic currents and that the spatial variation in ionic current density may explain AP differences. Regional variation in atrial ionic currents may play an important role in atrial arrhythmia generation and may present opportunities for improving antiarrhythmic drug therapy. (Circ Res. 1998;83:541-551.)

Key Words: ion channel ■ cardiac arrhythmia ■ atrial fibrillation ■ action potential duration ■ regional heterogeneity

The potential importance of heterogeneity in atrial refractoriness for the maintenance of atrial fibrillation (AF) has long been recognized,1 and increased refractoriness heterogeneity appears to play a significant role in a variety of experimental models2–6 and clinical populations7,8 with AF. Standard microelectrode experiments have demonstrated a potential substrate for atrial refractoriness heterogeneity, in terms of regional variability in action potential morphology.9–11 The most detailed findings have been reported in the dog, with consistent differences in action potential morphology and duration noted between the crista terminalis and several other right atrial regions.9,10 In the rabbit, action potential differences between the right and left atrial roofs11 and between the crista terminalis and pectinate muscles12 appear to involve differences in transient outward K+ current (Ito). Indirect evidence is compatible with regional variability in the importance of the rapid delayed rectifier K+ current (IKr) in the canine right atrium.13 Recent studies have pointed to variable regional expression of a variety of cloned ion channels in the ferret right atrium.14 Virtually no data are available regarding the ionic mechanisms underlying regional differences in canine atrial action potential morphology. Such information would be relevant because of the importance of refractoriness heterogeneity in canine AF models2,3,6 because of evidence for a role of regional differences in sensitivity to the IKr blocker dofetilide in the response to the drug of experimental atrial flutter,15 and because of the similarity between the properties of a variety of ionic currents in the canine atrium and those in human atrial cells.15–17 We therefore performed the present study to establish in canine right atrial tissues (1) whether the regional action potential differences previously reported in multicellular preparations5,10 are also present in single cells isolated from corresponding regions, (2) whether cells from different regions have different ionic current properties, and (3) whether the ionic current differences are consistent with the regional differences observed in action potential properties.

Materials and Methods

Cell Isolation

Adult mongrel dogs of either sex weighing 24.9±3.7 kg (n=19) were anesthetized with sodium pentobarbital (30 mg/kg IV), and their hearts were quickly removed and immersed in Tyrode’s solution at room temperature. All solutions used for dissection and perfusion were equilibrated with 100% O2. Single atrial cells were isolated by arterial perfusion with enzyme-containing solutions as described previously.15 The right coronary artery was cannulated,
and the right atrium was dissected free and perfused with Tyrode’s solution at 37°C for 5 minutes until the effluent was clear of blood. Any leaks from arterial branches were stopped by ligation with silk thread to ensure adequate perfusion. The tissue was then perfused at a constant rate of 12 mL/min with Ca2+-free Tyrode’s solution for 20 minutes, followed by 40 minutes of perfusion with the same solution containing collagenase (100 U/mL, type CLSII, Worthington Biochemical) and 0.1% BSA (Sigma Chemical Co). A small piece of tissue from each of 4 well-perfused regions (crista terminalis, appendage, right atrium, and the right ventricle) was minced, and the cells were superfused at 3 mL/min, initially with Ca2+-containing Tyrode’s solution and then the cells were superfused at 3 mL/min, initially with Ca2+-free Tyrode’s solution and then the cells were superfused at 3 mL/min, initially with Ca2+-containing Tyrode’s solution and then the cells were superfused at 3 mL/min, initially with Ca2+-free Tyrode’s solution for 20 minutes, followed by 40 minutes of perfusion with the same solution containing collagenase (100 U/mL, type CLSII, Worthington Biochemical) and 0.1% BSA (Sigma Chemical Co). A small piece of tissue from each of 4 well-perfused regions (crista terminalis, appendage, pectinate muscles, CT, crista terminalis, and AVR, AV ring). Anatomical landmarks are sinoatrial node (SAN), superior vena cava (SVC), inferior vena cava (IVC), and right ventricle (RV).

Solutions
The standard Tyrode’s solution contained (mmol/L) NaCl 136, CaCl2 2, KCl 5.4, MgCl2 0.8, NaH2PO4 0.33, dextrose 10, and HEPES 10 (pH 7.4 adjusted with NaOH). This solution was used for cell isolation and as the extracellular solution for action potential studies and was modified as indicated below when specific currents were studied. Experiments to study action potentials and all ionic currents except for the ultrarapid delayed rectifier K+ current (IKur.d) were performed at 35°C with the use of a Peltier-effect device (N.B. Datyner). The standard Tyrode’s solution contained (mmol/L) NaCl 136, CaCl2 2.0, KCl 20, MgCl2 1.0, NaH2PO4 0.33, dextrose 10, and HEPES 10 (pH 7.4 adjusted with CsOH). In studies of IKur.d, 2 mmol/L ryanodine was added to suppress IKur,d, which could otherwise overlap with and contaminate IKur.d. The pipette solution for IKur.d recording contained (mmol/L) CsCl 20, cesium aspartate 110, MgCl2 5, EGTA 10, GTP 0.1, MgATP 5, and Na2-phosphocreatine 5 (pH 7.4 adjusted with CsOH).

Data Acquisition
The whole-cell patch-clamp technique was used to record ionic currents in the voltage-clamp mode, and action potentials were recorded in current-clamp mode. Borosilicate glass electrodes (outer diameter, 1.0 mm) were filled with pipette solution and connected to a patch-clamp amplifier (Axopatch 1-D, Axon Instruments). Electrodes with tip resistances of 1 to 2 MΩ were used to record whole-cell currents, and tip resistances were 3 to 5 MΩ when action potentials were recorded. Action potentials were elicited by 2-ms twice-threshold pulses and were recorded at 0.1, 1, and 2 Hz. Action potential duration (APD) stabilized within 15 action potentials at each frequency, and steady-state APD was measured to 20% of the peak amplitude. Only cells in which action potentials were stable for at least 20 minutes were used for analysis. Action potential measurements were begun 5 minutes after cell rupture. Voltage command pulses were generated by a 12-bit digital-to-analog converter controlled by pClamp software (Axon). Recordings were low pass-filtered at half the sampling frequency. Data were sampled at rates varying from 2 to 25 kHz (with sampling at 10 to 25 kHz used for the action potential and the rapidly activating currents, ICa,L, ICa,B, and ICa,P, and sampling at 2 kHz used for the slower currents, ICa,L and ICa,P) and then stored on the hard disk of an IBM-compatible computer.

Liquid junction potentials (2 to 8 mV) were zeroed before formation of the membrane-pipette seal in Tyrode’s solution. Junction potentials between the bath and pipette solution averaged ±10 mV and were corrected for action potentials only. Mean seal resistance for cells from each region is shown in Table 1, along with cell dimensions measured with a calibrated graticule in the microscope eyepiece. Several minutes after seal formation, the membrane was ruptured by gentle suction to establish the whole-cell configuration. The series resistance (Rseries) was estimated by dividing the time constant obtained by fitting the decay of the capacitive transient by the calculated membrane capacitance (the time integral of the capacitive response to 5-mV hyperpolarizing steps from a HP of −60 mV, divided by the voltage drop) and was electronically compensated. Before Rseries compensation, the decay of the capacitive surge was expressed by a single exponential having a time constant of 532±36 μs (cell capacitance, 73.2±4.9 pF; n=82). Precompensation Rseries values averaged 7.5±0.4 MΩ. After compensation, the time constant was reduced to 112±8 μs (cell capacitance, 71.2±2.7 pF), and Rseries was reduced to 1.5±0.1 MΩ. Currents rarely exceeded 2 nA, and the maximum voltage drop across the Rseries did not exceed 3 mV. Input resistance was measured with the use of a series of 5-mV hyperpolarizations from −60 mV, as the slope of the line relating voltage change to resulting current flow. Cells from the crista terminalis

Figure 1. Schematic diagram of canine right atrium. Areas from which cells were isolated are indicated: APG, appendage; PM, pectinate muscles; CT, crista terminalis; and AVR, AV ring. Anatomical landmarks are sinoatrial node (SAN), superior vena cava (SVC), inferior vena cava (IVC), and right ventricle (RV).
Among the four regions, averaging overall differences among action potentials in different "average" action potentials illustrate quantitatively the major by digitally averaging all action potentials in each region. The cells from the region of the AV ring. Figure 2E was created prominent phase 1, with the major difference between the two voltage. Both pectinate and AV ring cells had a large and long plateau. Appendage cells showed the greatest variability duration and commonly showed a "spike and dome" with a plateau (ie, relatively positive plateau in morphology but had a preponderance of cells with a small

Phase 1 amplitude was quantified in each cell as the voltage difference between the peak of the overshoot and the end of rapid phase 1 repolarization and averaged 49±2 mV in crista cells, 51±1 mV in pectinate muscle cells, 54±2 mV in AV ring cells, and 39±1 mV in appendage cells (P<0.001 versus each of the other regions). Plateau voltage was quantified as the most positive voltage after the end of phase

<table>
<thead>
<tr>
<th>TABLE 1. Properties of Myocytes From Different Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crista Terminalis</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Length, ( \mu \text{m} )</td>
</tr>
<tr>
<td>Width, ( \mu \text{m} )</td>
</tr>
<tr>
<td>Surface area, ( \mu \text{m}^2 )</td>
</tr>
<tr>
<td>( C_m ), pF</td>
</tr>
<tr>
<td>( R_m ), GΩ</td>
</tr>
<tr>
<td>( R_{seal} ), GΩ</td>
</tr>
</tbody>
</table>

\( C_m \) indicates membrane capacitance; \( R_m \), input resistance; and \( R_{seal} \), seal resistance. Numbers in parentheses indicate the number of cells for each determination. Surface area was calculated, assuming right cylindrical geometry, as \( 2\pi RL \), where \( R \) is radius (1/2 width) and \( L \) is cell length.

"*" \( P<0.001 \) for significance of difference between crista and other regions; † \( P<0.01 \) for crista vs pectinates and \( P<0.02 \) for crista vs appendage and AV ring.

were larger and had greater membrane capacitance compared with those from other regions (Table 1). Therefore, all current values are represented as current densities (ie, normalized to capacitance). Cells with significant leak currents were rejected, and leakage compensation was not applied.

To ensure representativity of voltage-clamp data, similar numbers of cells from each heart were studied with each protocol (ie, the cells were distributed evenly across dogs). Furthermore, to ensure that interanimal variability did not bias results from various regions, similar numbers of cells from each region were studied from each heart, and the same measurements were made for each region within a given heart.

**Statistical Analysis**

Group data are presented as mean±SEM unless otherwise stated. Nonlinear curve fitting was performed with the Clampfit routine in pCLAMP (Chebyshev algorithm). Statistical comparisons among groups were performed by ANOVA. If significant effects were indicated by ANOVA, a t test with the Bonferroni correction or a Dunnett test was used to evaluate the significance of differences between individual mean values. A 2-tailed value of \( P<0.05 \) was taken to indicate statistical significance.

**Results**

**Regional Action Potential Heterogeneity**

Figure 2 shows all action potentials recorded from the crista terminalis (panel A, 41 cells), appendage (panel B, 41 cells), pectinates (panel C, 68 cells), and AV ring region (panel D, 51 cells). We have chosen to show all cells, rather than just "representative" examples, in order to provide a full appreciation of the variation among and within regions. Action potentials within the crista terminalis were the longest in duration and commonly showed a "spike and dome" with a long plateau. Appendage cells showed the greatest variability in morphology but had a preponderance of cells with a small phase 1 and high plateau (ie, relatively positive plateau voltage). Both pectinate and AV ring cells had a large and prominent phase 1, with the major difference between the two being the shorter plateau and more triangular appearance of cells from the region of the AV ring. Figure 2E was created by digitally averaging all action potentials in each region. The "average" action potentials illustrate quantitatively the major overall differences among action potentials in different regions.

Resting membrane potential was not significantly different among the four regions, averaging −73.5±0.3 (n=41), −71.2±0.3 (n=41), −72.8±0.1 (n=68), and −70.9±0.2 (n=51) mV in the crista, appendage, pectinates, and AV ring region, respectively. Action potential amplitude also showed no significant differences, averaging 126±3, 120±3, 123±2, and 119±3 mV, respectively, at 1 Hz. Mean values of APD at various frequencies are shown for all groups of dogs in Table 2. APD was significantly longer in cells from the crista compared with cells from the other 3 areas. Appendage and pectinate muscle cell APDs were not significantly different, but APDs in both areas were substantially greater than in cells near the AV ring at 0.1 and 1 Hz.

Phase 1 amplitude was quantified in each cell as the voltage difference between the peak of the overshoot and the end of rapid phase 1 repolarization and averaged 49±2 mV in crista cells, 51±1 mV in pectinate muscle cells, 54±2 mV in AV ring cells, and 39±1 mV in appendage cells (P<0.001 versus each of the other regions). Plateau voltage was quantified as the most positive voltage after the end of phase

![Figure 2](http://circres.ahajournals.org/content/543/1/801)

**Figure 2.** Action potentials in different regions. A to D, All action potentials recorded are shown for CT (n=41 cells) (A), APG (n=41 cells) (B), PM (n=68 cells) (C), and AVR (n=51 cells) (D). E, Action potentials obtained by digitally averaging all recordings from each respective region.
and was significantly smaller in AV ring cells than in other regions. The voltage-dependent activation of $I_{Ca}$ was fitted by a Boltzmann relation, with a half-maximal activation voltage of $-11.5\pm0.8$, $-12.3\pm0.4$, $-10.3\pm0.7$, and $-10.9\pm0.8$ mV in crista, appendage, pectinate, and AV ring regions, respectively ($n=10$ cells/group, $P=NS$). The voltage-dependent activation of $I_{Ca}$ was determined by dividing peak current during depolarizing test pulses by the driving force (calculated as the difference between test potential and the $I_{Ca}$ reversal potential from the plateau and the end of phase 1). Spike-and-dome morphologies were seen in 35 of 41 crista cells (85%; mean amplitude, $3.3\pm0.4$ mV), and 0 of 51 AV ring cells. 

### Figure 3. A to D, Typical recordings of $I_{Ca}$ from CT (A), APG (B), PM (C), and AVR (D). E, Mean±SEM $I_{Ca}$ density-voltage relations for each region ($n=25$ cells/region). Voltage protocol (240-ms pulses to voltage indicated at 0.1 Hz) is shown in inset. $**P<0.001$ vs APG and PM cells.

### Table 2. APD in Cells From Different Regions of Canine Right Atrium

<table>
<thead>
<tr>
<th>Region</th>
<th>CT</th>
<th>APG</th>
<th>PM</th>
<th>AVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD50</td>
<td>0.1 Hz</td>
<td>$186\pm8$</td>
<td>$120\pm2$</td>
<td>$103\pm3$</td>
</tr>
<tr>
<td></td>
<td>1 Hz</td>
<td>$157\pm5$</td>
<td>$118\pm2$</td>
<td>$98\pm2$</td>
</tr>
<tr>
<td></td>
<td>2 Hz</td>
<td>$120\pm4$</td>
<td>$85\pm2$</td>
<td>$49\pm2$</td>
</tr>
<tr>
<td>APD95</td>
<td>0.1 Hz</td>
<td>$328\pm13$</td>
<td>$210\pm7$</td>
<td>$245\pm4$</td>
</tr>
<tr>
<td></td>
<td>1 Hz</td>
<td>$270\pm10$</td>
<td>$180\pm3$</td>
<td>$190\pm3$</td>
</tr>
<tr>
<td></td>
<td>2 Hz</td>
<td>$219\pm7$</td>
<td>$155\pm2$</td>
<td>$152\pm3$</td>
</tr>
</tbody>
</table>

$APD_{50}$ and $APD_{95}$ indicate action potential duration to 50% and 95% repolarization, respectively; CT, crista terminalis; APG, appendage; PM, pectinate muscle; and AVR, AV ring. Results are mean±SEM in milliseconds for 41 CT cells, 41 APG cells, 68 PM cells, and 51 AVR cells. $*P<0.05$, $†P<0.01$, and $‡P<0.001$ vs APG cells at same frequency; $§P<0.05$, $‖P<0.01$, and $¶P<0.001$ vs PM cells at same frequency.
nential time constants averaging 27.8±2.9, 33.2±3.1, 29.2±2.6, and 32.2±3.4 ms in crista, appendage, pectinate, and AV ring regions, respectively (n=10 cells/group, P=NS). The frequency dependence of \( I_{\text{Ca}} \) was tested with a train of fifteen 200-ms pulses from \(-70\) to \(+10\) mV, with current during the last pulse of the train normalized to first-pulse current. Mean data from 10 cells/group (Figure 4D) show that \( I_{\text{Ca}} \) frequency dependence had no regional differences.

**Inward Rectifier K\(^+\) Current, \( I_{\text{K1}} \)**

Figure 5 (panels A to D) shows representative \( I_{\text{K1}} \) recordings from one cell in each region, as elicited by 300-ms pulses from an HP of \(-40\) mV to voltages ranging from \(-120\) to \(-20\) mV. To separate \( I_{\text{K1}} \) from potential contaminating currents, recordings were obtained before and after the addition of 0.5 mmol/L \( \text{Ba}^{2+} \) to the superfusate. Figure 5 shows \( \text{Ba}^{2+} \)-sensitive currents obtained by digital subtraction of currents after \( \text{Ba}^{2+} \) application from those before \( \text{Ba}^{2+} \) application. Mean current-voltage relations are shown in panel E. No significant regional differences in \( I_{\text{K1}} \) density were observed; eg, mean current densities at \(-120\) mV averaged \(-5.6±0.3, -5.1±0.5, -5.3±0.5, \) and \(-5.0±0.4 \text{pA/pF} \) in crista, appendage, pectinate, and AV ring regions, respectively (n=25 cells in each group). The lack of regional variation in \( I_{\text{K1}} \) is consistent with the lack of any regional differences in the resting membrane potential.

**Regional Differences in \( I_{\text{to}} \) Density**

Figure 6 (panels A to D) shows typical recordings of \( I_{\text{to}} \) from cells in each region, as determined with the use of 100-ms pulses from an HP of \(-80\) mV (0.1 Hz). Current amplitude was measured as the difference between peak and end-pulse steady-state current. There were no obvious differences in current time course, but currents tended to be smaller in cells from the appendage (panel B). Figure 6E shows mean \( I_{\text{to}} \) density as a function of test potential in each region (n=25 cells for each determination). There were no significant differences in \( I_{\text{to}} \) density among cells from crista, pectinates, and the AV ring region; however, \( I_{\text{to}} \) densities were approximately half as great in appendage cells compared with the other regions.

The voltage dependence and kinetics of \( I_{\text{to}} \) were analyzed in various atrial regions, as shown in Figure 7. Voltage-dependent inactivation was studied with a 1000-ms prepulse to various test potentials from an HP of \(-80\) mV, followed by
a 100-ms test pulse to +60 mV. The voltage dependence of activation was obtained from the current-voltage relation determined as shown in Figure 6, with changes in driving force corrected by dividing each current by the difference between test potential and the mean reversal potential of $I_{To}$ tail currents recorded after a 3-ms pulse to +50 mV (in the presence of 5 mmol/L TEA to inhibit $I_{Kur.d}$). The $I_{To}$ reversal potential averaged −74±4, −73±3, −74±3, and −73±3 mV in crista, appendage, pectinate, and AV ring cells, respectively (n=5 for each). Mean±SEM data for 10 cells/region are shown in Figure 7A, along with best-fit Boltzmann relations, and indicate no regional differences in $I_{To}$ voltage dependence. The half-activation voltage averaged 12.9±1.0, 13.1±1.1, 12.3±1.2, and 12.7±1.0 mV in crista, appendage, pectinate, and AV ring regions, respectively, and the half-inactivation voltage averaged −19.9±1.8, −21.5±2.3, −22.6±2.1, and −18.5±2.0 mV.

The rapidity of $I_{To}$ activation was assessed by fitting current activation by a third-order monoeponential relation, and inactivation kinetics were analyzed by fitting a biexponential relation to the time course of current decay during a depolarizing step. The time course of $I_{To}$ activation and inactivation, as shown in Figure 7B by mean±SEM results obtained in 10 cells/group, did not show any significant interregional differences. $I_{To}$ recovery kinetics were assessed with the use of paired 150-ms pulses from an HP to +50 mV with varying interpulse intervals (Figure 7C). The recovery of $I_{To}$ was well fitted by monoeponential functions. In 6 cells in which recovery time constants were measured at −80, −70, and −60 mV for each cell, the time constants averaged 20±2, 23±3, 21±3, and 24±3 ms at −80 mV, 23±2, 25±1, 25±3, and 26±2 ms at −70 mV, and 34±3, 34±3, 34±4, and 35±3 ms at −60 mV, respectively, in crista, appendage, pectinate, and AV ring cells (P=NS for interregional differences). The frequency dependence of $I_{To}$ was tested with a train of fifteen 100-ms pulses from −80 to +50 mV and showed no significant regional differences (Figure 7D, 10 cells/group).

**Delayed Rectifier K⁺ Currents**

Three types of delayed rectifier currents are present in dog atrium: classical $I_{Kr}$ and $I_{Ks}$ and an ultrarapid delayed rectifier ($I_{Kur.d}$) with properties that resemble those of Kv3.1 channels. Figure 8 shows representative currents elicited by 140-ms pulses from an HP of −50 mV to various test potentials, followed by repolarization for 60 ms to −30 mV to record tail currents, under conditions (including a prepulse to inactivate $I_{Va}$; see Materials and Methods for details) designed to isolate $I_{Kur.d}$. An HP of −50 and an 80-ms prepulse to +30 mV at 10 ms before the test pulse were used to suppress $I_{Va}$ and elicit selectively $I_{Kur.d}$ as previously described. Typical original recordings from each region are shown in panels A to D and have the rapid activation and large tail currents characteristic of $I_{Kur.d}$ with no obvious regional differences. Mean current-voltage relations for 25 cells/region, shown in panel E, indicate a lack of significant regional variation in the current.

Figure 9 shows an analysis of $I_{Ks}$ in all groups. Panels A to D show recordings obtained with the use of 3-s depolarizing pulses to various voltages, followed by a 1-s repolarization to...
−30 mV in the presence of 5 μmol/L E-4031 (to inhibit \( I_{\text{Kr}} \)) and 2 mmol/L 4-aminopyridine to inhibit \( I_{\text{Kur,d}} \) and \( I_{\text{to}} \). The overall form of original recordings was not different among the 4 groups, and mean time-dependent step current density-voltage relations (in 25 cells/group) showed no significant regional differences (Figure 9E). Similarly, there were no differences in tail current density (eg, after a step to 140 mV, values averaged 2.7±0.3 pA/pF in crista, appendage, pectinate, and AV ring regions, respectively).

\( I_{\text{Kr}} \) was recorded with the voltage protocol shown in Figure 10, with results obtained before and after the addition of 5 μmol/L E-4031 and \( I_{\text{Kr}} \) expressed as the E-4031–sensitive current. Representative \( I_{\text{Kr}} \) recordings from each region are shown in panels A to D of Figure 10. Larger currents were consistently recorded in cells from the AV ring region. Mean step current density-voltage relations (from 25 cells/group) are shown in Figure 10E and indicate that \( I_{\text{Kr}} \) was significantly larger in the AV ring region compared with the other 3 regions studied. Similar differences were noted in \( I_{\text{Kr}} \) tail current density; eg, after a step to 0 mV, tail current densities averaged 0.93±0.09 pA/pF in crista cells, 0.89±0.08 pA/pF in appendage cells, 0.91±0.09 pA/pF in pectinate muscle cells, and 1.61±0.18 pA/pF (\( P<0.001 \) versus other regions) in AV ring cells. Shown in Figure 10F is the voltage dependence of \( I_{\text{Kr}} \) activation, based on E-4031–sensitive tail currents at −30 mV after 3-s steps to the voltages indicated. Half-activation voltages averaged −29±1, −21±1, −10±1, and −9±1 mV in crista, appendage, pectinate, and AV ring cells, respectively (\( n=15 \) cells/group). Activation kinetics were also similar among regions; eg, at +10 mV, the activation time constants averaged 115±14, 104±15, 119±14, and 109±13 ms in crista, appendage, pectinate, and AV ring cells, respectively (\( P=\text{NS}, n=25 \) cells/group).

**Discussion**

The present findings indicate discrete differences in action potential morphology and duration in cells isolated from different regions of the canine right atrium. These differences correspond to previously published observations obtained with standard microelectrode techniques and were associated with clear variations in the densities of specific ionic currents, notably, \( I_{\text{Kr}}, I_{\text{Kur,d}}, \) and \( I_{\text{to}} \), whereas the densities of other currents did not show regional alterations.
Comparison With Previous Studies of Regional Action Potential Heterogeneity in the Atrium

Hogan and Davis\(^9\) described a variety of action potential morphologies recorded with standard microelectrodes in the canine right atrium. The action potentials they show from the crista terminalis, appendage, the “atrial roof” (tissue between pectinate muscles, corresponding to the region near the AV ring in our studies), and pectinate muscles are quite similar to our recordings from single cells isolated from corresponding regions. Spach et al\(^{10}\) showed a progressive decrease in plateau amplitude and APD from the crista terminalis to the pectinate muscles in multicellular canine right atrial preparations, with recordings quite similar to those we obtained in single cells from the crista and pectinate muscles, respectively. The similarity between our findings and previous reports in multicellular preparations points to the physiological relevance of our isolated cell preparation. The only other data regarding regional action potential variations in atrial tissue of which we are aware were obtained in the rabbit. Left atrial roof cells have a shorter duration and larger phase 1 than do right atrial cells,\(^{11}\) and as in the dog, cells from the crista have a longer action potential than do cells from pectinate muscles.\(^{12}\)

Ionic Mechanisms of Action Potential Heterogeneity

Over the past 10 years, there has been increasing awareness of the importance of action potential heterogeneity at the ventricular level.\(^{20}\) Transmural heterogeneity has been well studied in canine ventricular preparations, in which epicardial action potentials are characterized by a prominent phase 1 and spike-and-dome appearance,\(^{21}\) and midmyocardial (“M cells”) have a much longer APD.\(^{22}\) Similar observations have been made in guinea pigs,\(^{23}\) rabbits,\(^{24,25}\) and humans.\(^{26,27}\) The limited phase 1 amplitude in epicardial myocytes is due to a reduced $I_{to}$ density.\(^{21,28}\) The longer APD of M cells is due, at least in part, to a smaller $I_{Ks}$.$^{29,30}$ These spatial gradients in repolarization underlie several clinically important phenomena, including differences in rate-dependent repolarization of epicardium versus endocardium,\(^{31}\) the electrocardiographic J wave,\(^{32}\) triggered activity caused by quinidine and digitalis,\(^{33}\) and potentially arrhythmogenic responses to $I_{to}$ blockade,\(^{34}\) ATP-sensitive K$^+$ current activation,\(^{35}\) myocardial ischemia,\(^{36}\) and increased extracellular Ca$^{2+}$ concentration.\(^{37}\) Recent molecular work points to varying expression of Kv4.2 mRNA as the mechanism underlying transmural heterogeneity in $I_{to}$ expression.\(^{38}\)

The ionic basis of atrial action potential heterogeneity is poorly understood, and no data are available in the literature regarding the ionic mechanisms of atrial action potential heterogeneity in the dog. In the rabbit, differences in $I_{to}$ density appear to be particularly important,\(^{11,12}\) in keeping with the well recognized prominence of $I_{to}$ as a repolarizing...
Their greater APD and, in conjunction with a sizable initial variation we observed. The smaller recorded can explain much of the interregional action potential heterogeneity in the genesis and perpetuation of AF.41 There is increasing evidence suggesting a role for repolarization heterogeneity in the canine right atrium.44 This function may explain the consistent finding across species that action potentials are longer in the crista terminalis cells than in other right atrial regions.9–11 It remains to be determined whether other regional action potential properties have functional significance and whether they arise as a function of embryological origin, developmental factors, differential innervation, and hemodynamic factors, for exam-

Potential Limitations
We found significant interregional differences in action potential properties and ionic currents among cells isolated from different right atrial regions. The differences in ionic current densities were potentially able to explain the differences observed in mean action potential properties. On the other hand, within each region there was considerable variability in action potential properties of individual cells (eg, see Figure 2A to 2D). Action potential variability was greatest in cells from the atrial appendage (Figure 2B), consistent with previous observations of variability in action potential morphology in atrial cells from individual right atrial appendage preparations.43 Thus, although our findings explain overall interregional differences, we did not study the ionic mechanisms for the fine structure of action potential variability within atrial regions. It was not feasible within the context of the present study to assess the ionic mechanisms of intercell action potential variability within a given region, but this matter would be appropriate to pursue in future work.

The ionic current profiles of isolated cells are sensitive to variations in the nature and quality of the isolation. To minimize this potential source of variability, cells from each region were studied for each dog, action potentials were recorded from all isolates to ensure that ionic currents were recorded from preparations with comparable properties, and the same types of studies were performed on cells from all regions on each experimental day. The similarity between the action potential properties from cells in each region in our studies and previous observations with standard microelectrodes in multicellular preparations supports the validity of our observations.

Although we have described in detail the ionic substrate for spatial variability in canine right atrial action potential properties, the mechanisms responsible for creating regional differences and their physiological role remain to be determined. It has been suggested that the longer action potential of crista terminalis cells may help to direct impulses from the sinus node preferentially in the direction of the AV node.44 This function may explain the consistent finding across species that action potentials are longer in the crista terminalis than in other right atrial regions.5–11 It remains to be determined whether other regional action potential properties have functional significance and whether they arise as a function of embryological origin, developmental factors, differential innervation, and hemodynamic factors, for exam-

Potential Significance of Our Findings
There is increasing evidence suggesting a role for repolarization heterogeneity in the genesis and perpetuation of AF.41 The present study provides information regarding the ionic substrate for refractoriness heterogeneity in the canine right atrium. Although further work will be necessary to ascertain the applicability of our findings to humans, the present study provides potentially valuable insight into the mechanisms of atrial repolarization heterogeneity.

Understanding the ionic basis for refractoriness heterogeneity may provide new insight into mechanisms of antiarrhythmic drug action and help in the development of new approaches to developing antiarrhythmic drug therapy. Cha et al have shown that dofetilide is more effective than quinidine in suppressing atrial flutter in a dog model and acts at least in part by reducing the dispersion in atrial refractoriness. Restivo et al have shown that dofetilide increases the refractory period more in the lower right atrium of dogs with sterile pericarditis than in other regions. Our findings may explain this result in terms of the significantly larger $I_{Kr}$ that we found in lower right atrial cells (near the AV ring) compared with cells from other right atrial regions.

Figure 10. A to D, Typical recordings of $I_{Kr}$ (measured as E-4031-sensitive current) from CT (A), APG (B), PM (C), and AVR (D). E, Mean±SEM $I_{Kr}$ density-voltage relations for each region (n=25 cells/region). F, Mean±SEM voltage-dependence of $I_{Kr}$ activation based on tail current amplitudes after voltage steps indicated. Voltage protocol (3-s pulses to voltage indicated, followed by 1 s at −30 mV to record tail currents; protocol delivered at 0.1 Hz) is shown in the inset.

Current density and larger $I_{Kr}$ can explain the smaller plateau and $Kr$ can explain the smaller plateau and $Kr$ density in crista cells accounts for their longer duration of cells from the area near the AV ring.
ple. Recently developed immunolocalization techniques may be very helpful in studying the distribution of ion channel mRNA and proteins.45

We characterized the properties and density of $I_{\text{K}1}$ and several $K^+$ currents, and the variations observed are consistent with regional differences in action potential properties. We cannot, however, exclude a role of currents and transport mechanisms that we did not measure (e.g., $I_{\text{K-ATP}}$, Na$^+$, K$^+$-ATPase, Na$^+$-Ca$^{2+}$ exchanger, and Ca$^{2+}$-ATPase). The outward component of $I_{\text{K}1}$ was small and variable in our studies (Figure 5), as in previous work with atrial myocytes. Although no significant regional differences in $I_{\text{K}1}$ were noted, we cannot include small differences in the outward component of $I_{\text{K}1}$ that were beyond the resolution of our methods but could contribute to differences in repolarization. The degree to which cell coupling in the multicellular in situ heart would attenuate action potential variability is an important issue that cannot be addressed by the present study. Acetycholine decreases the space constant by increasing $K^+$ conductance,46 and vagal stimulation causes a substantial increase in atrial repolarization heterogeneity,47,48 which appears to be central in the clinically relevant ability of vagal stimulation to promote AF.2 It is conceivable that the ability of vagal stimulation to increase atrial repolarization heterogeneity and promote AF is related to its propensity to unmask spatial variations in action potential properties by reducing the influence of cell coupling on the spatial variability of repolarization.

Conclusions

We have shown that cells isolated from different regions of the canine right atrium have discrete action potential characteristics that are accompanied by different profiles of ionic current densities. The regional differences in ionic current expression account in large measure for regional action potential properties. These findings provide a basis for understanding regional heterogeneity in atrial repolarization, an important factor in the genesis and maintenance of atrial reentrant arrhythmias, and may lead to new insights into mechanisms of antiarrhythmic drug action and approaches to the development of novel antiarrhythmic therapies.

Acknowledgments

This study was supported by the Medical Research Council of Canada, the Quebec Heart Foundation, and the Fonds de Recherche de l’Institut de Cardiologie de Montréal. Dr Wang is a Canadian Heart Foundation Research Scholar, and L. Yue is the recipient of a Canadian Heart Foundation Research Studentship. The authors thank Nathalie Talbot and Mirie Levy for technical assistance and France Thériault for secretarial assistance with the manuscript.

References


Ionic Mechanisms of Regional Action Potential Heterogeneity in the Canine Right Atrium
Jianlin Feng, Lixia Yue, Zhiguo Wang and Stanley Nattel

Circ Res. 1998;83:541-551
doi: 10.1161/01.RES.83.5.541

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/83/5/541

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/