Regional heterogeneity of the action potential configuration and duration (APD) characterizes the ventricular myocardium in large mammals, including humans.1-3 A prominent notch shapes the typical spike-and-dome action potential of the epicardium and midmyocardium (M layer) but is absent in the endocardium. A relatively large transient outward current, containing a 4-aminopyridine–sensitive component (Ito1) and Ca2+–activated Cl− current, is mainly responsible for this notch. Another in vitro electrophysiological distinction is the longer APD of midmyocardium and its pronounced increase in response to slow pacing rates and class Ia and class III agents.1,4,5 These repolarization characteristics have been explained on the basis of a lesser contribution of the slowly activating component (Ik) of the delayed rectifier K+ current in M cells,4,6 whereas the rapidly activating component (Ikr) and the inward rectifier current (Ik1) appear similar in the 3 transmural layers.7

Only limited information is available on action potential and ionic differences in right ventricular (RV) versus left ventricular (LV) comparisons. A larger Ito1 in RV versus LV epicardial cells has been correlated with a larger notch in the former cell type.8 Because an interventricular comparison of K+ currents in M cells is lacking, we examined action potentials and the K+ currents Ito1, Ik, Ik1, and Ik1 in RV and LV M cells of the same adult canine hearts.

Methods

Sixteen mongrel dogs of either sex (26±1 kg) were anesthetized and received perioperative care as described previously.9 Thoracotomy was performed, and hearts (weight, 225±12 g) were quickly excised. RV and LV M cells were obtained by simultaneous cannulation of the left anterior descending and right coronary arteries.10 After ~30 minutes of collagenase perfusion, the epicardial surface layer was removed from both wedges until a depth of ≥3 mm was reached,4,7 and softened tissue samples were removed by pipette from the M layer underneath while contamination with the endocardium was avoided. Samples were gently agitated, filtered, and washed. Isolated myocytes were stored at room temperature in standard buffer solution.

The setup was built around an inverted microscope.9 Microelectrodes (standard glass) had resistances of 30 to 60 MΩ when filled with 3.0 mol/L KCl. Intracellular pacing was done at various cycle lengths (CLs). For the recording of ionic currents, we used the whole-cell variant of the patch-clamp technique. Patch pipettes (borosilicate glass) had resistances of 1.0 to 3.0 MΩ when filled with internal solution. Experiments were performed at 37°C. Cell capacitance, measured as almokalant-sensitive tail currents at −50 mV, and Ik1 were not different in the 2 ventricles.
current was inactivated by 10-ms prepulses to −45 mV. The voltage-clamp protocols are illustrated in Figures 1 and 2. \(I_{\text{TO1}}\) amplitudes were measured as peak amplitudes minus steady-state values at the end of the test pulses \(V_{\text{test}}\). For \(I_{\text{Kr}}\), we measured the tail currents on repolarization to −50 mV sensitive to almokalant (2 \(\mu\)mol/L; a specific \(I_{\text{Kr}}\) blocker). For \(I_{\text{Ks}}\), we measured steady-state values at the end of \(V_{\text{test}}\).
The standard-buffer solution used for the experiments was composed of (in mmol/L) NaCl 145, KCl 4.0, CaCl₂ 1.8, MgCl₂ 1.0, NaH₂PO₄ 1.0, glucose 11, HEPES 10, pH 7.4 with NaOH at 37°C. The patch-pipette solution contained (in mmol/L) potassium aspartate 125, KCl 20, MgCl₂ 1.0, MgATP 5, HEPES 5, EGTA 10, pH 7.2 with KOH.

Data are expressed as mean±SEM. Intergroup comparisons were made with the Student's t test for unpaired and paired data groups, after testing for the normality of distribution. Differences were considered significant if P<0.05.

Results

Action Potential Characteristics

Typical examples of RV and LV M action potentials are shown in Figure 1. Quantitative data are given in the Table. RV M cells had a more pronounced spike-and-dome configuration than LV M cells at fast and slow pacing rates. Both the action potential upstroke and plateau (phase 0 and phase

Figure 2. Quantitative data for I_{TO1}, I_{Ks}, I_{Kr}, and I_{K1} in RV versus LV M cells from 9 dogs. Current density-voltage relation (A; n_{RV}=12, n_{LV}=10) and steady-state inactivation (B; n_{RV}=9, n_{LV}=9) 1-second conditioning pulses, 300-ms V_{test}, 10-second interval) for I_{TO1}. Activation for I_{Ks} (C) and I_{Kr} tail currents (D) (n_{RV}=18 and n_{LV}=13). Current density-voltage relation for I_{K1} steady-state activation (E; n_{RV}=8 and n_{LV}=5). Asterisks indicate statistically significant differences in I_{TO1} and I_{Ks} current densities between RV and LV (P<0.05). V_{cond} indicates conditioning voltage.
and LV myocytes. Tail currents in 0 \([K^+]_o\) were best fitted by biexponential functions on repolarization to \(-10\) to \(-40\) mV and by monoeponential functions on more negative repolarizations.\((\sim 50\) to \(-80\) mV). At \(-20\) mV, time constants of the fast and slow components were \(228\pm25\) and \(1105\pm199\) ms in RV \((n=7)\) and \(278\pm35\) and \(1486\pm269\) ms in LV \((n=6)\), respectively; at \(-60\) mV, monoeponential time constants were \(99\pm16\) in RV and \(94\pm11\) in LV \((P=NS\) for all). 

\(I_{Kr}\) was quantified as the almokalant-sensitive tail-current portion measured by digital subtraction at \(-50\) mV in 4.0 mmol/L \([K^+]_o\) (Figure 2D). Activation showed saturation at conditioning voltages \(>20\) mV. Boltzmann fits to the data revealed \(V_{0.5}\) of \(2.9\pm1.0\) and \(4.3\pm2.5\) mV in RV and LV, respectively, while corresponding slope factors were \(6.2\pm2.1\) and \(5.3\pm0.8\) mV \((P=NS)\). \(I_{Ks}\) density was not different between RV and LV M cells. Voltage dependence and time course of \(I_{Ks}\) deactivation were also not different.

### Properties of \(I_{Ks}\)

Whole-cell recordings of \(I_{Ks}\) are shown in Figure 1. \(I_{Ks}\) rapidly activated and showed inactivation at the more negative voltages. In all cases, this current was fully inhibited in 0 \([K^+]_o\). There were no differences in the magnitude of \(I_{Ks}\) (initial minimal values as well as steady-state levels) between RV and LV M cells. Voltage dependence and time course of \(I_{Ks}\) deactivation were also not different.

#### Discussion

For interventricular comparisons of action potentials and \(K^+\) currents, we isolated myocytes from the deep subepicardial layers of the RV and LV free wall of the same canine hearts. In both ventricles, these myocytes have been designated M cells on the basis of distinctive electrophysiological characteristics.\(^1\)-\(^6\) Our results show that action potentials have a deeper notch, a shorter duration, and less prolongation on slowing of the pacing rate in RV than in LV M cells. A longer APD in the LV versus RV has already been recorded in dogs, both in vitro\(^4,6\) and in vivo (in dogs with complete atrioventricular block).\(^9\) In 6 dogs with sinus rhythm (CL, 507±32 ms), we found endocardial monophasic APDs to be longer in LV than RV in all animals (219±6 versus 203±6 ms; \(P<0.05)\).\(^12\) Taken together, these data indicate that a larger LV than RV APD exists at normal heart rates and during bradycardia.

The presence of \(I_{Ko}\) and \(I_{Kr}\) was confirmed in M cells from the LV and was also demonstrated in RV M cells. Densities of \(I_{Ko}\) were similar in both ventricles. \(I_{Kr}\) density however, was significantly larger in RV, and this difference could explain, at least in part, why APD\(_{50}\) and APD\(_{95}\) were longer and why the APD/pacing CL relationship was steeper in LV than in RV M cells. Heterogeneity of \(I_{Kr}\) across the transmural LV wall has been linked to dispersion of repolarization and the danger of torsade de pointes.\(^4,6\) Our results on \(I_{Kr}\) (and \(I_{Ko}\)) suggest that arrhythmogenic electromotive gradients could also arise at the septal junction of the RV and LV.

In human ventricular myocytes, the presence of \(I_{Kr}\) and \(I_{Ko}\) has also been demonstrated.\(^13\) Interestingly, Li et al.\(^13\) made their observations in apparently undiseased RV myocytes of patients with left-sided heart failure. The finding of substantial amplitudes of \(I_{Ko}\) and \(I_{Kr}\), as well as the sensitivity of both

### Action Potential Characteristics of Canine RV and LV M Cells

<table>
<thead>
<tr>
<th>Pacing CL 500 ms</th>
<th>RV M Cells ((n=11))</th>
<th>LV M Cells ((n=15))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude, mV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 0</td>
<td>118±2*</td>
<td>123±2</td>
</tr>
<tr>
<td>Phase 1</td>
<td>92±5*</td>
<td>105±3</td>
</tr>
<tr>
<td>Phase 2</td>
<td>102±2*</td>
<td>108±2</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>−84±2</td>
<td>−84±1</td>
</tr>
<tr>
<td>APD(_{50}), ms</td>
<td>272±8*</td>
<td>294±7</td>
</tr>
<tr>
<td>APD(_{50}), ms</td>
<td>206±7*</td>
<td>236±6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pacing CL 4000 ms</th>
<th>RV M Cells ((n=11))</th>
<th>LV M Cells ((n=15))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude, mV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 0</td>
<td>113±2</td>
<td>118±2</td>
</tr>
<tr>
<td>Phase 1</td>
<td>71±6*</td>
<td>87±4</td>
</tr>
<tr>
<td>Phase 2</td>
<td>102±2</td>
<td>105±2</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>−84±2</td>
<td>−84±1</td>
</tr>
<tr>
<td>APD(_{50}), ms</td>
<td>366±17 (+35%)*</td>
<td>437±34 (+6%)*</td>
</tr>
<tr>
<td>APD(_{50}), ms</td>
<td>297±13 (+44%)*</td>
<td>379±32 (+61%)*</td>
</tr>
</tbody>
</table>

Microelectrode technique. Cells were obtained from 8 dogs. Amplitudes were measured as the voltage difference between resting membrane potential and peak of upstroke (phase 0), deepest level of notch (phase 1), and dome (phase 2). APD was measured at 95% (APD\(_{95}\)) and 50% (APD\(_{50}\)) of repolarization. Percentages in brackets indicate relative increase vs CL 500 ms. *\(P<0.05\) vs LV M cells.
components to their blockers (E-4031 and indapamide), may underscore the importance of these currents for human ventricular repolarization, as expected from the clinical response to class Ia and class III agents in patients and from molecular studies on K⁺ channels in human myocardial tissue.

Our finding of a large $I_{TO1}$ in RV M cells is in keeping with the prominent spike-and-dome morphology of the action potentials. Yan and Antzelevitch¹⁴ presented evidence that the distribution of $I_{TO1}$ across the canine ventricular wall is causally linked to the J wave of the ECG. The joint results of this and another study⁸ indicate that a large $I_{TO1}$-mediated notch can be found throughout most of the RV mass, which suggests that the contribution of the RV to the formation of the J wave on the ECG may be larger than previously assumed. Furthermore, this may have important consequences for our understanding of the Brugada syndrome. ST-segment elevation in the right precordial ECG leads of patients suffering from this disorder has been linked to the concept of “all-or-none repolarization” in the RV epicardium.¹⁵ If our data are applicable to patients, then the substrate predisposed to all-or-none repolarization may cover most of the RV transmural wall.

References

Repolarizing K+ Currents $I_{TO1}$ and $I_{Ks}$ Are Larger in Right Than Left Canine Ventricular Midmyocardium

Paul G. A. Volders, Karin R. Sipido, Edward Carmeliet, Roel L. H. M. G. Spätjens, Hein J. J. Wellens and Marc A. Vos

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