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

Paul G.A. Volders, MD; Karin R. Sipido, MD, PhD; Edward Carmeliet, MD, PhD; Roel L.H.M.G. Späjtens, BS; Hein J.J. Wellens, MD, PhD; Marc A. Vos, PhD

Background—The ventricular action potential exhibits regional heterogeneity in configuration and duration (APD). Across the left ventricular (LV) free wall, this is explained by differences in repolarizing $K^+$ currents. However, the ionic basis of electrical nonuniformity in the right ventricle (RV) versus the LV is poorly investigated. We examined transient outward ($I_{TO1}$), delayed ($I_{Ks}$ and $I_{Kr}$), and inward rectifier $K^+$ currents ($I_{K1}$) in relation to action potential characteristics of RV and LV midmyocardial (M) cells of the same adult canine hearts.

Methods and Results—Single RV and LV M cells were used for microelectrode recordings and whole-cell voltage clamping. Action potentials showed deeper notches, shorter APDs at 50% and 95% of repolarization, and less prolongation on slowing of the pacing rate in RV than LV. $I_{TO1}$ density was significantly larger in RV than LV, whereas steady-state inactivation and rate of recovery were similar. $I_{Ks}$ tail currents, measured at $-25$ mV and insensitive to almokalant (2 μmol/L), were considerably larger in RV than LV. $I_{K1}$, measured as almokalant-sensitive tail currents at $-50$ mV, and $I_{Kr}$ were not different in the 2 ventricles.

Conclusions—Differences in $K^+$ currents may well explain the interventricular heterogeneity of action potentials in M layers of the canine heart. These results contribute to a further phenotyping of the ventricular action potential under physiological conditions. (Circulation. 1999;99:206-210.)

Key Words: action potential ● myocytes ● ions ● potassium ● arrhythmia

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 ($I_{TO1}$) and Ca$^{2+}$-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 ($I_{Ks}$) of the delayed rectifier $K^+$ current in M cells,6,7 whereas the rapidly activating component ($I_{K1}$) and the inward rectifier current ($I_{Kr}$) 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 $I_{TO1}$ 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 $I_{TO1}$, $I_{Ks}$, $I_{Kr}$, and $I_{K1}$ 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.10 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 by hyperpolarizing steps from ~60 mV, was similar in RV (n=27) and LV (n=25) M cells, being 226±12 and 226±11 pF, respectively ($P$=NS). L-type Ca$^{2+}$ current was blocked with nifedipine (5 μmol/L). Na$^+$...
current was inactivated by 10-ms prepulses to −45 mV. The voltage-clamp protocols are illustrated in Figures 1 and 2. $I_{\text{T01}}$ amplitudes were measured as peak amplitudes minus steady-state values at the end of the test pulses ($V_{\text{test}}$). For $I_{\text{K1}}$, we measured the tail currents on repolarization to −50 mV sensitive to almokalant (2 μmol/L; a specific $I_{\text{K1}}$ blocker). For $I_{\text{K1}}$, we measured the almokalant-insensitive tail currents on repolarization to −25 mV.

Figure 1. $K^+$ currents $I_{\text{T01}}$ and $I_{\text{K1}}$ are larger in RV than LV M cells isolated from the same normal dog heart, whereas $I_{\text{K1}}$ is similar. $I_{\text{T01}}$ was activated by $V_{\text{test}}$ from −40 up to 70 mV from a holding potential of −70 mV (interval, 10 seconds). Shown are the traces of −10 to 70 mV. $I_{\text{K1}}$ was recorded in the presence of almokalant; we applied depolarizations from −20 up to 70 mV followed by repolarizations to −25 mV. Shown are the traces of −70 to −140 mV. $I_{\text{K1}}$ was recorded during $V_{\text{m}}$ of −20 to −140 mV. Shown are the traces of −70 to −140 mV. Holding potential was −50 mV (interval, 3 seconds). Left horizontal bars indicate 0-pA level. Capacitances of RV and LV M cells used for illustrations are similar ($I_{\text{T01}}$: 247 and 226 pF; both $I_{\text{K1}}$ and $I_{\text{K1}}$: 249 and 244 pF). Action potentials (microelectrode technique; vertical scale bars indicate 0 to −50 mV) were recorded at pacing CLs of 500 and 4000 ms and illustrate that the difference of configuration and rate-dependent prolongation between ventricles is likely related to underlying $K^+$-current differences.
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.¹ Both the action potential upstroke and plateau (phase 0 and phase
Properties of $I_{\text{TO1}}$

$I_{\text{TO1}}$ activated at $V_{\text{res}} \approx -20 \text{ mV}$ in both ventricles, but amplitudes were significantly larger in RV than at $V_{\text{res}}$ (Figures 1 and 2A). A-aminopyridine (5 mmol/L) nearly completely suppressed $I_{\text{TO1}}$ in both cell types. Inactivation during the $V_{\text{res}}$ was best fitted with a single exponential function yielding similar time constants for RV and LV. The voltage dependence of $I_{\text{TO1}}$ steady-state inactivation (Figure 2B) was well described by a Boltzmann fit with half points ($V_{1/2}$) of $-52 \pm 0.6$ and $-50 \pm 0.5 \text{ mV}$ and slope factors of $6.8 \pm 0.6$ and $4.5 \pm 0.5 \text{ mV}$ in RV and LV, respectively ($P=\text{NS}$). Time-dependent recovery from inactivation was not different between the ventricles.

Properties of $I_{\text{Ks}}$ and $I_{\text{Kr}}$

$I_{\text{Ks}}$ tail currents were evaluated on repolarization to $-25 \text{ mV}$ with $I_{\text{Kr}}$ blocked by almokalant. Examples of current traces are shown in Figure 1. Pooled data are given in Figure 2C. There was no saturation of tail-current amplitudes. Voltage dependence of $I_{\text{Ks}}$ activation was similar for both cell types, but density was significantly larger in RV (0.72 $\pm 0.12 \text{ pA/pF}$) than in LV (0.32 $\pm 0.13 \text{ pA/pF}$) ($P<0.05$; depolarization to 50 mV). This difference persisted after increasing $I_{\text{Ks}}$ in K$^+$-free solution (0 [K$^+$]$_0$): 0.98 $\pm 0.21 \text{ pA/pF}$ in RV versus 0.58 $\pm 0.17 \text{ pA/pF}$ in LV. Deactivation proved similar in RV and LV myocytes. Tail currents in 0 [K$^+$]$_0$ were best fitted by biexponential functions on repolarization to $-10$ to $-40 \text{ mV}$ and by monoexponential functions on more negative repolarizations ($-50$ to $-80 \text{ mV}$). At $-20 \text{ mV}$, time constants of the fast and slow components were 228 $\pm 25$ and 1105 $\pm 199$ ms in RV ($n=7$) and 278 $\pm 35$ and 1486 $\pm 269$ ms in LV ($n=6$), respectively; at $-60 \text{ mV}$, monoexponential time constants were 99 $\pm 16$ in RV and 94 $\pm 11$ in LV ($P=\text{NS}$ for all).

$I_{\text{Kr}}$ was quantified as the almokalant-sensitive tail-current portion measured by digital subtraction at $-50 \text{ mV}$ in 4.0 mmol/L [K$^+$]$_0$ (Figure 2D). Activation showed saturation at conditioning voltages $>20 \text{ mV}$. Boltzmann fits to the data revealed $V_{1/2}$ of 2.9 $\pm 1.0$ and 4.3 $\pm 2.5 \text{ mV}$ in RV and LV, respectively, while corresponding slope factors were 6.2 $\pm 2.1$ and 5.3 $\pm 0.8 \text{ mV}$ ($P=\text{NS}$). $I_{\text{Kr}}$ density was not different between RV and LV M cells. Voltage dependence and time course of $I_{\text{Kr}}$ deactivation were also not different.

Properties of $I_{\text{K1}}$

Whole-cell recordings of $I_{\text{K1}}$ are shown in Figure 1. $I_{\text{K1}}$ rapidly activated and showed inactivation at the more negative voltages. In all cases, this current was fully inhibited in 0 [K$^+$]$_0$. There were no differences in the magnitude of $I_{\text{K1}}$ (initial minimal values as well as steady-state levels) between RV and LV throughout the voltage range tested (Figure 2E).

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 vitro4,8 and in vivo (in dogs with complete atrioventricular block).9 In 6 dogs with sinus rhythm (CL, 507 $\pm 32$ ms), we found endocardial monophasic APDs to be longer in LV than in RV in all animals (219 $\pm 6$ versus 203 $\pm 6$ 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 $I_{\text{Kr}}$ and $I_{\text{Ks}}$ was confirmed in M cells from the LV and was also demonstrated in RV M cells. Densities of $I_{\text{Kr}}$ were similar in both ventricles. $I_{\text{Ks}}$ density however, was significantly larger in RV, and this difference could explain, at least in part, why APD$_{\text{DAP}}$ and APD$_{\text{DAP}}$ were longer and why the APD/pacing CL relationship was steeper in LV than in RV M cells. Heterogeneity of $I_{\text{K1}}$ across the transmural LV wall has been linked to dispersion of repolarization and the danger of torsade de pointes.8,10 Our results on $I_{\text{Ks}}$ and $I_{\text{Kr}}$ suggest that arrhythmic mechanisms could also arise at the septal junction of the RV and LV.

In human ventricular myocytes, the presence of $I_{\text{Kr}}$ and $I_{\text{Ks}}$ has also been demonstrated.13 Interestingly, Li et al13 made their observations in apparently undiseased RV myocytes of patients with left-sided heart failure. The finding of substantial amplitudes of $I_{\text{Ks}}$ and $I_{\text{Kr}}$ as well as the sensitivity of both
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 $I_{\text{TO1}}$ in RV M cells is in keeping with the prominent spike-and-dome morphology of the action potentials. Yan and Antzelevitch$^{14}$ presented evidence that the distribution of $I_{\text{TO1}}$ across the canine ventricular wall is causally linked to the J wave of the ECG. The joint results of this and another study$^8$ indicate that a large $I_{\text{TO1}}$-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.

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