Transmural Heterogeneity of the Action Potential Configuration in the Feline Left Ventricle

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There are M cells in the canine, rabbit, guinea pig, and human left ventricle (LV), but it is not known if they are present in the feline LV. Arterially perfused feline LV preparations were used for the recording of transmembrane action potentials from the epicardium (Epi), midmyocardium (M) and endomyocardium (Endo) under control conditions (n=12) and in the presence of I_{Ks} blocker (chromanol 293B: 10 μ mol/L, n=6) or I_{Kr} blocker (E-4031: 2 μ mol/L, n=6). The steady-state action potential duration at 90% repolarization and cycle length (APD₉₀/CL) relation was obtained and fitted by the hyperbolic function $APD_{90} = CL / [(a \times CL) + b]$. In control, the shortest and longest action potential duration (APD) were observed in Epi and M, respectively, and the APD₉₀/CL-relation curve was steeper in the M or Endo than in the Epi. Chromanol 293B prolonged APD in Epi, but not in M or Endo, resulting in no significant difference of the APD₉₀/CL-relation curve among the 3 regions. E-4031 markedly, but homogeneously, prolonged APD in all regions, giving rise to decreased transmural dispersion of repolarization. In conclusion, there exists an M cell layer with a longer APD than the Epi and Endo layers and there is transmural electrical heterogeneity in the feline LV; however, the response to I_{Kr} blocker is different from that of the canine LV probably because of species differences in the I_{Kr} and I_{Ks} . (Circ J 2003; 67: 449–454)

Key Words: ECG; Ion channel; Membrane potential; Myocytes

The regional differences in the electrophysiological properties of the ventricular myocardium have been well established.^{1,2} Sicouri and Antzelevitch were the first to describe a subpopulation of cells (M cells) in the subepicardial and midmyocardial layers of the canine ventricular wall.³ They suggested that the M cell was distinguished electrophysiologically from other cells by the disproportionate prolongation of its action potential duration (APD) in response to slowing of the stimulation rate and to APD prolonging agents such as blockers of the rapidly activating delayed rectifier potassium current (I_{Kr}). The weaker, slowly activating delayed rectifier potassium current (I_{Ks}) and larger late sodium current (I_{Na}) in the M regions are reported to contribute to the longer APD in M cells than in other cell types.⁴ Moreover, recent experiments using wedge preparations have shown that the electrophysiological heterogeneity across the ventricular wall produced the inscription of the normal and abnormal T wave and was responsible for the occurrence of ventricular tachyarrhythmias.^{5–9}

The presence of M cells has been shown in the deep layers of the canine, pig, rabbit, guinea pig, and human left ventricle (LV).^{3,10–13} The feline heart is commonly used in experimental studies examining the effect of myocardial ischemia,^{14–17} ventricular hypertrophy,^{18,19} and sympathetic and parasympathetic nerve activity^{20,21} on ventricular

action potentials. Although previous studies by Myerburg et al showed the ionic and cellular basis for the electrophysiological difference between epicardial and endocardial cells in the feline ventricle,^{22–24} its transmural heterogeneity of APD and the presence of M cell remains unknown. The purpose of this investigation was to assess the electrophysiological and pharmacological characteristics of epicardial, midmyocardial, and endocardial cells in the feline LV. The results indicated that transmural electrophysiological heterogeneity existed in the feline LV.

Methods

Arterially Perfused Wedge of Feline Ventricle

Cats weighting 2.0–4.0 kg were anesthetized with intraperitoneal pentobarbital (1.0 mg/kg). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4°C) Tyrode's solution containing 8.5 mmol/L $[K^+]$ and transmural wedges with dimensions of approximately 2×1×0.5 cm to 3×1×1 cm were dissected from the anterior wall of the LV. The tissue was cannulated via a branch of the left descending coronary artery with cardioplegic solution. The preparation was then placed in a small tissue bath and arterially perfused by a roller pump (Cole Parmer Instrument Co) with Tyrode's solution of the following composition (mmol/L): 129 NaCl, 4 KCl, 0.9 NaH₂PO₄, 20 NaHCO₃, 1.8 CaCl₂, 0.5 MgSO₄, and 5.5 glucose, buffered with 95% O₂ and 5% CO₂ (37±1°C). Perfusion pressure was maintained between 40 and 60 mmHg by adjusting the flow rate of the pump. The ventricular wedges were allowed to equilibrate in the tissue bath until electrically stable for 1 h and stimulated with bipolar electrodes applied to the endocardial surface.

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Fig 1. Transmembrane action potentials (AP) and a transmural ECG simultaneously recorded from a feline left ventricular wedge preparation under baseline conditions. The AP were recorded from the epicardial (Epi), longest midmyocardial (M), and endomyocardial (Endo) regions at a basic cycle length of 1,000 ms using floating glass microelectrodes. The briefest AP in the Epi coincided with the peak of the T wave in the ECG, whereas the longest action potential in the M region coincided with the end of the T wave.

Recording of ECG and Transmembrane Action Potentials (AP)

A transmural ECG was recorded with Ag–AgCl electrodes, which were placed in the Tyrode's solution bathing the preparation, 1.0 cm from the epicardial and endocardial surfaces (epicardial, positive pole). Transmembrane AP in the wedge preparations were recorded simultaneously from the epicardium, midmyocardium, and endomyocardium by the use of 3 separate intracellular floating microelectrodes (DC resistance 10–20 M Ω ; 2.7 mmol/L KCl) connected to a high-input impedance amplification system (World Precision Instruments). We recorded epicardial and endocardial AP from the epicardial and endocardial surfaces of the preparations at positions approximating the transmural axis of the ECG recording; the midmyocardial AP were recorded at the area between the epicardium and endocardium along the same axis. All amplified signals were digitized at 2 kHz using a 12-bit analog-to-digital converter and stored on a hard disk of a dedicated laboratory computer system, and analyzed using the original software of our laboratory. All APD measurements were reported at 90% repolarization (APD₉₀). Transmural dispersion of repolarization (TDR) was defined as the difference between the longest and shortest repolarization times (activation time + APD₉₀) of AP recorded across the wall (M–epicardial region). The recordings of ECG and transmembrane AP in the feline LV were similar to those reported using wedge preparations.^{5–9}

Study Protocol

Transmural ECG and transmembrane AP were recorded in the absence (control condition) and presence of the I_{Ks} blocker (chromanol 293B: 10 μ mol/L) or I_{Kr} blocker (E-4031: 2 μ mol/L). Then, we studied the relation between APD₉₀ and cycle length (CL) at steady state pacing of

Fig 2. Distribution of the action potential duration at 90% repolarization (APD₉₀) across the feline left ventricular wall at a cycle length (CL) of 1,000 ms under control conditions. Transmural distance at 0% and 100% represent the epicardium (Epi) and endomyocardium (Endo), respectively. Midmyocardial action potentials were recorded at the area between the epicardium and endocardium along the same axis. Data represent mean $\pm$ SEM (n=5).

2,000, 1,500, 1,000, and 500 ms.

Data Analysis

APD₉₀ measured at steady state was plotted as a function of pacing CL and these experimental points were accurately fitted ($r > 0.95$) by the hyperbolic relation introduced for this purpose by Elharrar et al:^{25,26}

$$APD_{90} = CL / [(a \times CL) + b]$$

In this equation, the coefficients 'a' and 'b' define the degree of APD₉₀ dependence on CL. The term 1/a represents the asymptotic value of APD₉₀ at an infinitely long CL (1/a = APD_{max}); that is, the one that would occur if an AP was stimulated in a quiescent fiber. Thus, APD_{max} is independent of rate, and is thought to reflect the intrinsic membrane properties that determine the time course of repolarization. The CL at which APD equals 50% of APD_{max} (CL₅₀) can be estimated from 'b' and APD_{max} as follows:

$$CL_{50} = b \times APD_{max}$$

Statistical Analysis

Estimation of the parameters describing the APD₉₀/CL relation at steady state was performed by a linear least square fitting between 1/APD₉₀ and 1/CL. The goodness of fit was tested by the correlation coefficient $r > 0.95$. Mean values of the variables among groups were tested using ANOVA. Individual post hoc comparisons were made using Bonferroni/Dunn test. Data are expressed as mean $\pm$ SD values, except for those shown in the Figs, which are expressed as mean $\pm$ SEM values. Significance was defined as a value of $p < 0.05$.

Results

AP Configuration and Distribution

Fig 1 illustrates a representative transmembrane AP simultaneously recorded from the epicardium, the longest midmyocardium (M region), and the endocardium together with a transmural ECG at the CL of 1,000 ms under control conditions. The AP of the epicardium and M region typically exhibited a distinct spike and dome morphology,

Fig 3. Transmembrane action potentials (AP) simultaneously recorded from the epicardial (Epi), longest midmyocardial (M), and endomyocardial (Endo) regions across the feline left ventricular wall at cycle lengths (CL) of 500, 1,000, and 2,000 ms under baseline (control) conditions (Panel A), after chromanol 293B (10 μ mol/L) (Panel B), and E-4031 (2 μ mol/L) (Panel C). Chromanol 293B slightly prolonged the action potential duration (APD) in the epicardial and endocardial regions only, not in the M region. E-4031 pronouncedly prolonged the APD in all regions.

whereas those of the endocardial cell lacked the dome and exhibited a negatively sloping phase-2. In comparison with the transmural ECG, repolarization of the briefest AP in the epicardium coincided with the peak of the T wave, whereas the longest AP in the M region coincided with the end of the T wave. The repolarization of the endocardium was intermediate between that of the epicardium and M region. Fig 2 shows the composite data ($n=5$) of the transmural APD distribution at a CL of 1,000 ms across the feline LV in control conditions. The APD₉₀ was briefest at the epicardium, gradually increased, and became longest at the subendocardium. These transmembrane AP configurations and the transmural APD distribution in our experiments were similar to those in canine ventricular wedge preparations.^{5-9,19}

Effects of I_{Ks} or I_{Kr} Blocker on APD/CL Relationship

Fig 3 illustrates a superimposed transmembrane AP recorded from the epicardial, M, and endocardial regions at CLs of 500, 1,000, and 2,000 ms (steady-state condition) in the control state and after chromanol 293B (10 μ mol/L) and E-4031 (2 μ mol/L). Slowing the stimulation rate prolonged the APD in the 3 regions in the control condition, after

Fig 4. Composite data of the relation between action potential duration measured at 90% repolarization (APD₉₀) and pacing cycle length (CL) in the epicardial (Epi), midmyocardial (M), and endomyocardial (Endo) regions of the feline left ventricular wall under baseline (control) condition ($n=12$) (Panel A), after chromanol 293B (10 μ mol/L) (Panel B), and E-4031 (2 μ mol/L) (Panel C). Continuous curves were computed from the hyperbolic fitting method. Data are mean $\pm$ SEM. * $p < 0.05$ vs. Epi, † $p < 0.05$ vs. Endo.

chromanol 293B and after E-4031.

Fig 4 and Table 1 show the composite data of the APD₉₀/CL relationship in the control state ($n=12$), and after chromanol 293B ($n=6$) and E-4031 ($n=6$). In control conditions, the APD_{max} was significantly larger in the M and endocardial regions than in the epicardium ($p < 0.01$), but CL₅₀ was not significantly different among the 3 regions (Table 1). Therefore, the APD₉₀/CL-relation curve was steeper in the M or endocardial region than in the epicardium under control conditions (Fig 4, Panel A). Chromanol 293B (10 μ mol/L) increased the APD_{max} in the epicardium ($p < 0.05$), but not in the M or endocardial region, resulting in no significant difference in APD_{max} among the 3 regions. Moreover, the CL₅₀ was not significantly different among the 3 regions after chromanol 293B. Therefore, chromanol 293B caused the APD₉₀/CL-relation curve homogeneously in the 3 regions (Fig 4, Panel B). On the other hand, E-4031 (2 μ mol/L) markedly increased the APD_{max} in all 3 regions, and it was larger in the M region than in the epicardium or endocardium ($p < 0.05$). The CL₅₀ was also prolonged after E-4031 ($p < 0.01$), but was not significantly different among the 3 regions. Therefore, E-4031 steepened the APD₉₀/CL-relation curve, especially in the M region (Fig 4, Panel C).

Effects of I_{Ks} or I_{Kr} Blockers on TDR

Fig 5 illustrates a superimposed transmembrane AP

Table 1 Parametric Description on the Steady-State APD₉₀/CL Relation

	Region	$a (\times 10^{-3}) (ms^{-1})$	$b^{\#}$	APD _{max} (ms)	CL ₅₀ (ms)
Control ($n=12$)	Epi	4.2 ± 0.37	0.58 ± 0.09	239 ± 22	140 ± 30
	M	$3.5 \pm 0.15^*$	0.57 ± 0.17	$285 \pm 13^*$	162 ± 49
	Endo	$3.7 \pm 0.28^*$	0.54 ± 0.13	$269 \pm 18^*$	145 ± 37
Chromanol 293B ($n=6$)	Epi	$3.7 \pm 0.49^{\dagger}$	0.67 ± 0.22	$277 \pm 43^{\dagger}$	194 ± 96
	M	3.4 ± 0.43	0.68 ± 0.11	302 ± 44	209 ± 64
	Endo	3.5 ± 0.45	0.68 ± 0.14	289 ± 43	201 ± 76
E-4031 ($n=6$)	Epi	$2.5 \pm 0.19^{\ddagger}$	0.51 ± 0.11	$407 \pm 33^{\ddagger}$	$207 \pm 49^{\ddagger}$
	M	$2.2 \pm 0.16^{\ddagger*}$	0.56 ± 0.08	$461 \pm 34^{\ddagger*}$	$260 \pm 41^{\ddagger}$
	Endo	$2.4 \pm 0.23^{\ddagger}$	0.50 ± 0.17	$420 \pm 39^{\ddagger}$	213 ± 88

Epi, epicardium; M, midmyocardium; Endo, endomyocardium. $^{\#}$ Dimensionless. * $p < 0.05$ vs. Epi, $^{\dagger}p < 0.05$ vs. control, $^{\ddagger}p < 0.01$ vs. control.

Fig 5. Superimposed transmembrane action potentials recorded from the epicardial (Epi) and M regions, and the transmural ECG at cycle length of 1,000 ms before (control) and after chromanol 293B (Panel A) and E-4031 (Panel B).

recorded from the epicardial and M regions and transmural ECG at BCL of 1,000 ms before and after chromanol 293B (Fig 5, Panel A) and E-4031 (Fig 5, Panel B). Chromanol 293B slightly prolonged the APD in the epicardium, but not in the M region, resulting in decreasing TDR without any change of QT interval. On the other hand, E-4031 prolonged APD markedly, but homogeneously, in the epicardial and M regions, resulting in increasing QT interval, but decreasing TDR. Fig 6 shows the composite data of the APD₉₀/CL relationship in the epicardial, M, and endocardial regions and TDR before and after chromanol 293B or E-4031. Chromanol 293B slightly prolonged the APD₉₀ in the epicardium (Fig 6, Panel A), but not in the M and endocardial regions (Fig 6, Panels B and C), resulting in decreasing TDR (Fig 6, Panel D). On the other hand, E-4031 markedly and homogeneously prolonged APD₉₀ in the 3 regions (Fig 6, Panels A–C), and decreased TDR (Fig 6, Panel D).

Discussion

We have shown that the electrophysiological characteristics and pharmacological responsiveness were transmurally heterogeneous in the perfused feline LV. We found a subpopulation of cells in the midmyocardium with a longer APD, moderate phase-1 notch and steeper APD/CL-relation curve under control conditions and in the presence of an I_{Kr} blocking agent. These characteristics are similar to those of M cells previously reported in other mammalian species, including humans, thereby suggesting transmural electrical heterogeneity in the feline LV.

Transmural AP Heterogeneity

The regional difference in AP configuration between the epicardial, M, and endocardial cells has been well demonstrated in canine ventricular wedge, tissue and myocyte preparations.^{3–9,27–29} In the feline LV, several experiments have demonstrated the characteristics and differences in AP between the epicardium and endocardium^{14,15,22,23} but not of the midmyocardium. The present study is, to our best knowledge, the first to show transmural heterogeneity of the electrophysiological characteristics and pharmacological responses in the intact feline LV. The shortest and longest APD in the feline LV were observed in the epicardium and subendocardium (M region), respectively, consistent with those in the canine wedge preparation.^{5–9,19} Moreover, the AP in the feline epicardium and M region had a

Fig 6. Composite data of the relation between action potential duration measured at 90% repolarization (APD₉₀) and cycle length (CL) under baseline (control) conditions (n=12), after chromanol 293B (n=6) and after E-4031 (n=6) in the epicardial (Epi) (Panel A), mid-myocardial (M) (Panel B), and endocardial (Endo) (Panel C) regions, and the transmural dispersion of repolarization (TDR) (Panel D) across the feline left ventricular wall. Continuous curves in Panels A–C were computed from the hyperbolic fitting method. Data are mean $\pm$ SEM. * $p < 0.05$ vs. Control, † $p < 0.05$ vs. 293B.

phase-1 notch, whereas that in the endocardium lacked the notch and exhibited a negatively sloping phase-2. Furthermore, the correlations between the AP and transmural ECG in the feline wedge were similar to those in previous canine experiments. These findings suggest that the feline ventricular AP configuration and distribution across the transmural surface are similar to those in the canine ventricle. There exists the M-cell like layer in the feline LV, but its behavior is not identical to other animal species.

Characteristics of M Cells in the Feline LV

Marked AP prolongation with slowing of the stimulation rate is one of the characteristics of the M cell in the canine LV.^{1–3} The APD₉₀ in the M region is longer than that in the epicardium and endocardium, but the APD₉₀/CL-relation curve under control conditions in the present study was not so much steeper in the M region than in the epicardium or endocardium. It is conceivable that intact cell-to-cell coupling in the wedge preparation might cause the APD₉₀/CL-relation curve to be flatter in the M region compared with isolated tissue preparations and myocytes.²⁷

Another characteristic of the feline M cell is its unique sensitivity to I_{Kr} blocking agents. In the previous canine wedge experiments, I_{Kr} block with d-sotalol produced greater prolongation of the APD in the M cell than in the epicardial and endocardial cells, resulting in an increase of TDR.⁵ In the present feline LV preparation, the APD₉₀/CL-relation curve became much steeper in all regions after I_{Kr} block with E-4031, resulting in a homogeneously prolonged APD and decreased TDR, which is in striking contrast to the results in canine wedge preparations. The reason for this difference is the differential distribution and ratio of I_{Kr} and I_{Ks} across the feline LV.

Ionic Currents

In the canine ventricle, the I_{Kr} current is homogeneous across the myocardial layers, but the I_{Ks} current was weaker in the M region than in the epicardium and endocardium,

contributing to the longer APD in the M cells.⁴ In contrast, the distribution of I_{Kr} and I_{Ks} across the feline LV has never been demonstrated. Chapula reported the existence of 2 components of the delayed rectifier K^+ current (I_{Kr} and I_{Ks}) in cat ventricular myocytes.³⁰ Follmer et al reported that I_{Kr} blockade by flecainide and E-4031 almost completely reduced the delayed rectifier K^+ current (I_K);²⁴ although they did not show which cell type from the transmural layers was examined. Moreover, Furukawa et al showed that total I_K was greater in the epicardium than in the endocardium in the feline ventricle, and that the regional I_K difference contributed to the shorter APD in the epicardium than in the endocardium.²² These findings suggest that the I_K in the feline LV is mainly dependent on I_{Kr} , which is consistent with our data showing a prominent prolongation of APD₉₀ in all 3 layers with E-4031, but only a slight prolongation of APD with chromanol 293B.

Moreover, I_{Ks} block with chromanol 293B preferentially prolonged APD in the epicardium (206 ± 23 to 230 ± 20 ms) rather than in the M region (247 ± 28 to 247 ± 26 ms) and endocardium (234 ± 15 to 245 ± 21 ms) at the CL of 1,000 ms. I_{Kr} block with E-4031 also preferentially prolonged APD in the epicardium (206 ± 23 to 343 ± 26 ms) rather than in the M region (252 ± 28 to 366 ± 24 ms) and endocardium (234 ± 15 to 351 ± 24 ms) at the CL of 1,000 ms. These findings suggested that both I_{Kr} and I_{Ks} are larger in the epicardial cells than in the M and endocardial cells in the feline heart. Recently, we showed that I_{Kr} block based on I_{Ks} block markedly prolonged the QT interval and TDR, and induced early afterdepolarizations and torsades de pointes in the feline LV wedge preparation.³¹ Therefore, we suggest that these characteristics of the feline heart (I_{Kr} dependent) can be used to mimic drug-induced QT prolongation and torsades de pointes.

Study Limitations

The effects of the I_{Kr} and I_{Ks} blockers on the prolongation of APD in each region suggested a regional difference in I_{Kr} and I_{Ks} densities, but we did not directly record the I_{Kr} and I_{Ks} density of isolated feline ventricular myocytes in each region. It is difficult to explain all the characteristics and behaviors of the AP in the feline LV using only the I_{Kr} and I_{Ks} . The contribution of other currents (especially I_{Na} , I_{CaL} , I_{to} , I_{K1}) needs to be taken into account.

Our data showed a longer APD in the M region after E-4031, but we have never observed early afterdepolarization and torsades de pointes in this preparation, which might be because (1) we only paced at CL of 500–2,000 ms, and (2) E-4031 markedly, but homogeneously, prolonged APD, resulting in reducing TDR.

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