A Subpopulation of Cells With Unique Electrophysiological Properties in the Deep Subepicardium of the Canine Ventricle

The M Cell

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Recent studies have shown that canine ventricular epicardium and endocardium differ with respect to electrophysiological characteristics and pharmacological responsiveness and that these differences are in large part due to the presence of a prominent transient outward current $I_{\text{o}}$ and a spike-and-dome morphology of the action potential in epicardium but not endocardium. In attempting to quantitate these differences and assess their gradation across the ventricular wall, we encountered a subpopulation of cells in the deep subepicardial layers with electrophysiological characteristics different from those of either epicardium or endocardium. These cells, which we have termed M cells, display a spike-and-dome morphology typical of epicardium but a maximal rate of rise of the action potential upstroke that is considerably greater than that of either epicardium or endocardium. Using the restitution of the amplitude of phase 1 of the action potential as a marker for the reactivation of $I_{\text{o}}$, we showed M cells to possess a prominent 4-aminopyridine-sensitive $I_{\text{o}}$ with a reactivation time course characterized by two components with fast and slow time constants. The rate dependence of action potential duration of M cells was considerably more accentuated than that of epicardium or endocardium and more akin to that of Purkinje fibers (not observed histologically in this region). Phase 4 depolarization was never observed in M cells, not even after exposure to catecholamines and/or low $[K^+]_o$. In summary, our study presents evidence for the existence of a unique subpopulation of cells in the deep subepicardium of the canine left and right ventricles with electrophysiological features intermediate between those of conducting and myocardial cells. Although their function is unknown, M cells may facilitate conduction in epicardium and are likely to influence or mediate the manifestation of electrocardiographic J waves, T waves, U waves, and long QT intervals and contribute importantly to arrhythmogenesis. Circulation Research 1991;68:1729–1741

Recent studies have shown that ventricular epicardium (EPI) and endocardium (ENDO) differ with respect to electrophysiological characteristics and pharmacological responsiveness in canine and feline hearts. The presence of a prominent transient outward current ($I_{\text{o}}$) and a spike-and-dome morphology of the action potential in EPI but not ENDO appear to be responsible for many of the observed differences.

In recent studies designed to quantitate these differences and assess their gradation across the ventricular wall, we encountered a subpopulation of cells in the deep subepicardial layers with electrophysiological characteristics different from those of either EPI or ENDO. These cells, which we have termed M cells, display a spike-and-dome morphology typical of EPI but a maximal rate of rise of the action potential upstroke ($V_{\text{max}}$) that is considerably greater than that of either EPI or ENDO. Moreover, the rate dependence of action potential duration (APD) of cells in the M region is much more accentuated than that of EPI or ENDO but more akin to that of Purkinje fibers. Purkinje fibers, however, are not observed histologically in this region. The present study was designed to provide an initial characterization of this unique subpopulation of cells.
Materials and Methods

Experimental Preparation

ENDO (papillary muscles or ventricular trabeculae), EPI, deep subepicardial, or transmural strips (−2.0×1.5×0.1–0.2 cm) were isolated from the ventricles of hearts removed from anesthetized (30 mg/kg sodium pentobarbital) mongrel male dogs. The preparations were obtained by razor blade shavings (Dermatome Power Handle No. 3293 with cutting head No. 3295, Davol Simon, Cranston, R.I.) made parallel or perpendicular (transmural) to the surface of the ventricular free wall. The use of the terms ENDO and EPI in the present study refer to the myocardial cells on the respective surfaces of the ventricular wall, representing the outermost subendocardial and subepicardial layers.

The preparations were placed in a tissue bath and allowed to equilibrate for at least 2 hours while superfused with an oxygenated (95% O₂−5% CO₂) Tyrode’s solution (37±0.5°C, pH 7.35). The composition of the Tyrode’s solution was (mM) NaCl 129, KCl 4, NaH₂PO₄ 0.9, NaHCO₃ 20, CaCl₂ 1.8, MgSO₄ 0.5, and d-glucose 5.5.

Action Potential Recordings

The tissues were stimulated at basic cycle lengths (BCLs) ranging from 300 to 5,000 msec using field stimulation or rectangular stimuli (1–3-msec duration, 2.5 times diastolic threshold intensity) delivered through silver bipolar electrodes that were insulated except at the tips.

Transmembrane potentials were recorded from one or more sites using glass microelectrodes filled with 2.7 M KCl (10–20 MΩ DC resistance) connected to a high input–impedance amplification system (World Precision Instruments, New Haven, Conn.) Recordings were obtained from 1) sequential thin (1–1.5-mm) slices shaved from EPI to ENDO and 2) transmural slices. Amplified signals were displayed on an oscilloscope (Tektronix, Beaverton, Ore.) and photographed on a 35-mm kymographic camera (Grass Instrument Co., Quincy, Mass.). Vmax was measured with a differentiator adjusted for linearity within the range of 50–500 V/sec.

Because we found no major differences between the characteristics of the deep subepicardial cells obtained by transmural slices or slices parallel to the ventricular wall, we have grouped these together in the presentation of the results. Care was taken to avoid transitional cells in obtaining data representative of ventricular ENDO. In the case of papillary muscles, recordings were always obtained from the apical region, known to be devoid of Purkinje fibers.

Restitution of action potential characteristics was determined using single test pulses (S₂) delivered after every 10th basic beat (S₁). The S₁-S₂ coupling interval was increased progressively from the end of the refractory period until the next basic beat. The effective refractory period was defined as the longest S₁-S₂ interval at which S₂ failed to elicit a propagated response.

Drugs

4-Aminopyridine (4-AP, Sigma Chemical Co., St. Louis) was dissolved in distilled water and made soluble by warming to yield a stock solution of 0.5 M. The pH of the stock solution was adjusted to 7.4 with HCl.

Statistics

Statistical analysis was performed using analysis of variance coupled with Scheffe’s or Tukey’s procedure and linear regression fitting techniques.

Results

Action Potential Morphology

The presence of Iᵥ is known to contribute to the manifestation of a prominent early repolarization phase (phase 1) and thereby to the spike-and-dome morphology of action potentials recorded from canine and feline ventricular EPI. The lack of a prominent Iᵥ in ENDO is believed to account for the lack of a conspicuous phase 1 and spike-and-dome configuration in this tissue. These characteristics of transmembrane activity recorded from EPI and ENDO are illustrated in Figure 1 together with activity recorded from midmyocardial tissues. The four tracings represent recordings obtained from serial dermatome shavings of the free wall of the left ventricle.
ventricle. The preparations were stimulated at a BCL of 800 msec. The spike-and-dome configuration, most accentuated in EPI, attenuates in succeeding layers, suggesting the contribution of a progressively less intense $I_o$ as one moves from EPI to ENDO.

The difference between the phase 1 amplitude of a basic beat elicited by stimulation at relatively slow rates and the phase 1 amplitude of the earliest premature beat ($\Delta$ phase 1 amplitude) has been suggested to provide a reasonable measure of the intensity of $I_o$ in EPI. In an attempt to quantitate the contribution of $I_o$ to the electrical activity of cells spanning the ventricular wall, we examined these characteristics in cells from tissue segments isolated by successive shavings of the ventricular wall. Figure 2 plots the $\Delta$ phase 1 amplitude as a function of the distance of the recording site (expressed as a percentage of the total width of the ventricular wall) from the EPI surface in tissues isolated from three different hearts (left ventricle). A gradual diminution of $\Delta$ phase 1 amplitude is observed as we progress from EPI to ENDO, suggesting a gradual decrease in the contribution of $I_o$. Qualitatively similar results were obtained in the right ventricle.

**Characteristics of Deep Subepicardial Cells: M Cells**

Aside from differences in the early phases of the action potential, transmembrane activity recorded from cells in the deep subepicardial and midmyocardial layers of the ventricular wall appears, at first glance, to be no different from that of EPI or ENDO. Closer inspection, however, reveals that the electrophysiological characteristics of some cells within these layers are quite unique. Figure 3 illustrates responses recorded from cells in EPI, ENDO, and deep subepicardial (M region, 3.5 mm from EPI surface) layers of the canine left ventricle. At a BCL of 300 msec (Figure 3, left), APDs of responses recorded at the three transmural sites were similar. Under these conditions, the only distinction between M region and EPI activity was the much greater $V_{\text{max}}$

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** $\Delta$ Phase 1 amplitude (see inset) as a function of the transmural depth of the recording. The graph plots $\Delta$ phase 1 amplitude as a function of the distance of the site of recording (expressed in percent) from the epicardial surface in three different left ventricular preparations. Each symbol represents a different experiment. Inset: Transmembrane recording, obtained from a left ventricular epicardial preparation, showing the basic ($S_1$) and earliest premature ($S_2$) beats. The basic cycle length was 2,000 msec.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Transmembrane activity recorded from epicardial (Epi), deep subepicardial (M Region), and endocardial (Endo) preparations isolated from a canine left ventricle. BCL, basic cycle length. The M cell was recorded 3.5 mm from the Epi surface.
in the M cell. When BCL was increased to 2,000 msec (Figure 3, right), another striking distinction became evident; APD at 90% repolarization (APD90) prolonged remarkably in cells from the M region but only modestly in cells from EPI or ENDO (at BCL of 2,000 msec, APD90 was 356 msec in M region, 252 msec in EPI, and 245 msec in ENDO).

These salient features of M cells were observed in 19 of 19 left ventricular preparations studied and in five of five right ventricular preparations. In the left ventricle they were recorded at depths of 1.5–5.2 mm from the EPI surface, whereas in the right ventricle they appeared at depths of 1.2–2.3 mm.

Figure 4 illustrates activity recorded from an M cell (1.5 mm from EPI surface) in the right ventricle together with activity of EPI and ENDO. At slow stimulation rates, APD and Vmax of the M cell were considerably greater than those recorded from EPI or ENDO (Vmax was 565 V/sec in the M cell, 195 V/sec in ENDO, and 182 V/sec in EPI).

Table 1 summarizes the results of 24 experiments in which action potential parameters were measured in EPI, M region, and ENDO preparations isolated from the left ventricle. The APD of M cells was significantly longer than that of either EPI or ENDO at a BCL of 2,000 msec but not at a BCL of 300 msec. Vmax of M cells was significantly greater than that of EPI or ENDO at slow as well as rapid stimulation rates. Another distinction was the more negative resting membrane potential of M cells when compared with EPI or ENDO (M cells, -90.6±3.3 mV; EPI, -86.6±4.4 mV; and ENDO, -87.2±3.7 mV).

### Table 1. Action Potential Parameters of Epicardial, M Region, and Endocardial Cells Recorded From Canine Left Ventricle Preparations at Basic Cycle Lengths of 300 and 2,000 msec.

<table>
<thead>
<tr>
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<th>BCL of 300 msec</th>
<th>BCL of 2,000 msec</th>
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<tr>
<td></td>
<td>(n=12)</td>
<td>(n=17)</td>
</tr>
<tr>
<td>Rest potential</td>
<td></td>
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</tr>
<tr>
<td>EPI</td>
<td>85.7±4.4</td>
<td>89.6±3.2*</td>
</tr>
<tr>
<td>M region</td>
<td></td>
<td></td>
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<tr>
<td>Rest potential</td>
<td></td>
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<td>ENDO</td>
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Values are mean±SD; n=24 experiments. Epi, epicardial cells; M region, deep subepicardial cells; Endo, endocardial cells; BCL, basic cycle length; APD90, action potential duration at 90% repolarization; APDmax, action potential duration at 90% repolarization; Vmax, maximal rate of rise of action potential upstroke. Significance was determined by analysis of variance coupled with Scheffe’s test.

\( p<0.05 \) vs. Epi; \( \ddagger p<0.01 \) vs. M region; \( \ddagger\ddagger p<0.01 \) vs. Epi.

Only the difference between M and EPI was statistically significant.

Although the electrophysiological characteristics of cells from the M region are similar to those of Purkinje fibers, M cells can be distinguished from Purkinje cells by the lack of phase 4 depolarization, even in the presence of catecholamines and/or low [K+]o. Phase 4 depolarization was not observed in transmural preparations (n=2) or parallel shavings at the level of the M cells (n=4) exposed to low [K+]o (0.5–2 mM) or in transmural (n=2) or parallel shavings (n=3) exposed to norepinephrine (10^-6–10^-5 M) and different [K+]o (2–4 mM).

To rule out the possibility of an artifact, we examined the electrophysiological characteristics of preparations obtained by 1) dermatome shavings of different widths made parallel to the free wall of the left and right ventricles, 2) dermatome shavings made perpendicular to the wall, and 3) high-speed drill transmural core samples. M cells were found in all cases and were localized to distinct layers or regions of the ventricular wall as noted above. Once recovered (1–3 hours), the shaved preparations showed stable electrophysiological characteristics for many hours.
Rate Dependence of Action Potential Duration in Epicardium, Endocardium, and M Cells Under Steady-State Conditions

The presence of I_{in} in canine ventricular EPI is known to influence the rate dependence of APD in that tissue. Deceleration is usually attended by a marked accentuation of the spike-and-dome morphology of the EPI response due in large part to the relatively slow recovery kinetics of I_{in}. Accentuation of the spike-and-dome configuration contributes to APD prolongation by increasing the delay between the first and second action potential upstrokes. Therefore, the APD-rate relation is generally steeper in EPI than in ENDO, a tissue largely devoid of I_{in}. These characteristics of EPI and ENDO are illustrated in Figures 5 and 6 and contrasted with those of M cells. A shift from a BCL of 300 msec to a BCL of 5,000 msec produces a marked accentuation of the spike-and-dome configuration in EPI. Phase 1 becomes more prominent, and the peak plateau is achieved later, usually reaching a more positive potential. In contrast, no change is observed in the early phases of the ENDO action potential. Although the EPI action potential is briefer than that of ENDO at rapid stimulation rates, the converse is true at slow rates. As a result, the APD-rate relations of the two tissues cross over at a BCL between 600 and 1,000 msec. Whereas EPI generally shows a small progressive prolongation of APD at BCLs longer than 1,000 msec, ENDO does not.

In contrast, action potentials recorded in the M region displayed far greater changes in APD in response to changes in rate (Figures 5A and 6A). Deceleration-induced accentuation of the spike-and-dome morphology of the M cell action potential, although similar to that observed in EPI, appears to contribute little if any to the rate dependence of APD in these cells. The APD-rate relations recorded from M cells of both right (Figure 5B) and left (Figure 6B) ventricles are considerably steeper when compared with the APD-rate relations obtained from ENDO and EPI preparations from the same heart. In the right ventricle, an increase of the BCL from 300 to 5,000 msec caused a 125-msec increase in the APD_{90} of the M cell but an increase of only 57 msec in EPI and 47 msec in ENDO (Figure 5). In the left ventricle, a similar deceleration produced an even more dramatic prolongation of APD of the M cell (255 msec, Figure 6).

Figure 7A summarizes the results of five experiments performed in transmural preparations from the right ventricle. At rapid stimulation rates (300–500 msec), the APD of responses recorded simultaneously from EPI, M region, and ENDO are fairly similar. With progressive slowing of the stimulation rate, APD prolongation was considerably more pronounced in the M cell than in either EPI or ENDO. A progressively larger dispersion of repolarization times developed between the M cells and all other cells in the tissue at BCLs longer than 500 msec. Over

**Figure 5.** Rate dependence of the action potential of epicardial (Epi), deep subepicardial (M Region), and endocardial (Endo) cells in the canine right ventricle. Panel A: Transmembrane activity recorded from Epi, Endo, and M cells at basic cycle lengths (BCLs) of 300, 1,000, 2,000, and 5,000 msec (steady-state conditions). Panel B: Graphic representation of action potential duration at 90% repolarization (APD_{90}) as a function of BCL.
the range of frequencies studied, APD prolonged an average of 115.5±15.6 msec in the M cells but only 50.1±7.9 msec in EPI and 35.5±8.1 msec in ENDO. Rate-dependent changes in the effective refractory period paralleled the changes in APD.

Figure 7B plots the composite results obtained from 24 preparations (38 recording sites) isolated from the left ventricle in the form of strips shaved either parallel or perpendicular to the surface of the free wall. Deep subendocardial cells were recorded from slices located 1.5–3.1 mm from the ENDO surface; M cells (deep subepicardial cells) were recorded from slices located 1.5–5.2 mm from the EPI surface. As in the right ventricle, a much steeper APD–rate relation is observed in cells from the M region, as compared with the other three regions. It is noteworthy that the deceleration-induced prolongation of APD was considerably more pronounced in the left ventricle than in the right (compare Figures 7A and 7B). In the left ventricle, the APD90 of the M cells increased by an average of 203±42.9 msec after a change of BCL from 300 to 5,000 msec.

**Figure 7B.** (A) Rate dependence of the action potential duration (APD) at 90% repolarization (APD90) as a function of BCL. (B) Graphic representation of action potential duration at 90% repolarization (APD90) as a function of BCL.

**Restitution of Action Potential Parameters in Epicardial and M Cells**

The results thus far presented indicate important distinctions between M cells and other cells in the ventricular wall with respect to rate-dependent changes in APD under steady-state conditions but indicate similarities in the rate dependence of phase 1 amplitude between M cells and EPI. Since the determinants of APD and phase 1 amplitude are known to differ between steady-state and non-steady-state stimulation conditions, we next evaluated the characteristics of restitution of these parameters in EPI and M cell preparations.

The two curves shown in Figure 8A depict the restitution of APD in an M cell and an EPI cell from the same transmural preparation isolated from the left ventricular free wall. The APD90 of premature beats elicited once after every 10th basic beat (at BCL of 2,000 msec) are plotted as a function of the diastolic interval (interval between the full repolarization of the basic beat and the upstroke of the premature beat). Once again, we observe a much steeper relation for the M cell (top curve in Figure 8A) than for the EPI cell. In both cells, APD continues to increase throughout diastole, and the restitution curves are well fitted by biexponential curves, implying two components of recovery with short and long time constants.

A previous study has suggested that the slow progressive recovery of Ito is in large part responsible for the slow component of restitution of APD in EPI. To assess the characteristics of Ito recovery in these two tissues, we measured the restitution of Δ phase 1 amplitude, which in previous studies has been suggested to provide a reasonable measure of the intensity and kinetics of recovery of Ito in EPI.

Figures 8B and 8C graphically illustrate the recovery of phase 1 amplitude in the same preparations from which the results of Figure 8A were obtained. Each panel shows a semilogarithmic plot of the difference between the phase 1 amplitude of the basic beat (at BCL of 2,000 msec) and that of premature beats introduced at progressively longer S1–S2 intervals (see insets, Figures 8B and 8C). The Δ phase 1 amplitude is plotted on a semilogarithmic scale so that an exponential decline provides a straight line. Two exponential components with disparate time constants are apparent in both cell types. These fast
and slow components have been shown to represent the calcium-activated and 4-AP-sensitive components of $I_{\text{Ca}}$, respectively. It is noteworthy that the recovery of both components is slower in the M cell when compared with EPI (Table 2). The restitution of $\Delta$ phase 0 amplitude in both cells (not shown) could also be well fitted by a biexponential with time constants very similar to those obtained for the recovery of $\Delta$ phase 1 amplitude.

**Effect of 4-Aminopyridine on the Recovery of Phase 1 Amplitude in M Cells**

Figure 9 shows the effects of 4-AP, an $I_{\text{Ca}}$ blocker, on the recovery of $\Delta$ phase 1 amplitude in an M cell from the left ventricle. Under control conditions (panel A), two exponential components were apparent: a fast component with a time constant of 96 msec and a slow component with a time constant of 745 msec. The addition of 1 mM 4-AP (panel B) produced a diminution in the spike-and-dome morphology of the action potential, causing a decrease in the maximal values of the slow and fast components of $\Delta$ phase 1 amplitude recovery to 66% and 35% of their control values, respectively. An increase of 4-AP concentration to 5 mM (panel C) completely abolished the slow component and further reduced the intensity of the fast component to 20.7% of its control value. 4-AP caused no major change in the time constant of either component.

In two experiments in which 4-AP (1 mM) largely abolished the spike-and-dome morphology of the EPI action potential, we obtained a direct measure of the 4-AP-sensitive components by evaluating differences between the phase 1 amplitudes recorded before and after 4-AP. Figure 10 illustrates the results of one such analysis (same preparation as in Figure 9). In this format, the 4-AP-sensitive component(s) is represented by the voltage difference of phase 1 amplitude recorded before and 30 minutes after the introduction of 4-AP (Figure 10, top inset). The difference voltage is plotted on a logarithmic scale as a fraction of the maximal phase 1 amplitude difference measured at a BCL of 2,000 msec. The recovery process is thus evaluated and plotted as a function of the diastolic interval. The bottom inset illustrates the method used to derive the time constants. The results are well fitted by two exponential components with time constants similar to those observed using the method illustrated in Figure 9. The data confirm the presence of a relatively slow reactivation process for $I_{\text{Ca}}$ in M cells.

**Discussion**

Our results provide evidence in support of the existence of a distinct and unique subpopulation of cells in the deep subepicardial layers of the canine ventricle. These cells display characteristics common to both myocardial (spike-and-dome morphology, absence of phase 4 depolarization) and specialized conducting cells (higher $V_{\text{max}}$, steeper APD-rate relation). Unlike specialized conducting cells, however, they show no phase 4 depolarization, not even in the presence of catecholamines and low $[K^+]_o$. We have...
TABLE 2. Slow and Fast Time Constants for Recovery of Phase 1 Amplitude Changes as an Estimate of the Time Constants of Reactivation of the Slow and Fast Components of the Transient Outward Current: M Cell Versus Epicardial Cell

<table>
<thead>
<tr>
<th>Time constant</th>
<th>M cell (n=5)</th>
<th>Epicardium (n=5)</th>
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<tbody>
<tr>
<td>τ₁</td>
<td>656±173</td>
<td>368±94*</td>
</tr>
<tr>
<td>τ₂</td>
<td>117±16</td>
<td>43±9†</td>
</tr>
</tbody>
</table>

Values are mean±SD. M cell, deep subepicardial cell; τ₁, slow time constant; τ₂, fast time constant. *p<0.05 and †p<0.01 vs. M cell.

Figure 8. Restitution of action potential duration at 90% repolarization (APD₉₀) and phase 1 amplitude in an epicardial (Epi) and deep subepicardial (M) cell recorded simultaneously from a transmural preparation. Basic cycle length was 2,000 msec. Panel A: APD₉₀ is plotted as a function of the diastolic interval (interval between the full repolarization of the basic beat and the upstroke of the premature beat). Panels B and C: Recovery of phase 1 amplitude in Epi and M cells. τ, time constant. Each panel shows a semilogarithmic plot of the difference between the phase 1 amplitude of the basic beat (basic cycle length of 2,000 msec) and that of premature beats introduced at progressively longer S₁-S₂ intervals (see inset). The Δ phase 1 amplitude (●) is plotted on a logarithmic scale so that an exponential decline provides a straight line. Curves were fitted by eye.

... these M cells and the layers from which they arise as the M region.

Although cells with Purkinje-like action potential morphologies are commonly encountered in the atria (e.g., specialized conduction cells or plateau fibers), it is well established that subendocardial Purkinje fibers do not penetrate more than 2–3 mm into the wall of the canine ventricle. The location of the M cells in the deep subepicardium, within 1.5 mm of the EPI surface, excludes the possibility of their being Purkinje or transitional cells.

A recent preliminary report by Hoyt and Saffitz of morphologically distinct myocytes in the deep subepicardium and midmyocardium of the canine ventricle provides correlative evidence in support of the existence of this population of cells. It is noteworthy that these cells were histologically characterized as possessing features common to both specialized conducting and myocardial cells. These “putative intramural conduction cells” contain T tubules like other ventricular myocytes but are otherwise structurally similar to subendocardial conduction (transitional) cells. In a recent collaborative effort, we provided these investigators with samples of our preparations in which a microelectrode was broken to identify the region at which M cells were recorded. They were able to trace the microelectrode tract to a region in the deep subepicardium that contained cells with ultrastructural features of intramural conduction cells (personal communication, J. Saffitz, October 1990).

The continuity between the Purkinje system and ventricular myocardium has been the subject of numerous studies (see Reference 12 for review). All point to the Purkinje network as the final portion of the cardiac ventricular conduction system. No anatomic or other evidence is currently available to suggest a connection or link of M cells in the deep subepicardium with the subendocardial Purkinje system.

Transient Outward Current in Ventricular Muscle

A prominent contribution of Iₒ to transmembrane activity has been shown to exist in several cardiac tissues, most recently in canine1,20,21 and feline4,22 EPI preparations. The presence of a prominent Iₒ in canine ventricular EPI, but not ENDO, was shown to...
account for the appearance of action potentials with a pronounced spike-and-dome morphology in EPI but not ENDO. Our results suggest that an I_{\text{Ca}^{2+}}^-mediated spike-and-dome morphology is also a characteristic of M cells. In preliminary studies designed to map the distribution of these cells, we found that they extend to the midmyocardium. The gradual diminution of the spike-and-dome morphology of the action potential found across the ventricular wall (Figure 2) is likely due to a progressive decrease in the contribution of I_{\text{Ca}^{2+}}^- to transmembrane activity. Our findings are consistent with those of Tseng and coworkers, who reported that -64% of cells enzymatically dissociated from transmural left ventricular preparations display a spike-and-dome configuration.

I_{\text{Ca}^{2+}}^- is believed to be predominantly carried by K^+ ions and shows voltage-dependent activation, inactivation, and reactivation; a calcium-activated component has been reported in a number of studies (see Reference 1). By using the restitution of the amplitude of phase 1 of the action potential as a marker for the reactivation of I_{\text{Ca}^{2+}}^-, two exponential processes have previously been delineated in canine ventricular EPI: 1) a slow component that recovers with a time constant of -400 msec and is largely abolished by the I_{\text{Ca}^{2+}}^- blocker 4-AP (1–5 mM) and 2) a fast component with a time constant of -50 msec that is diminished by 4-AP but is also inhibited by ryanodine and by Sr\(^{2+}\) replacement of \(\text{Ca}^{2+}\), interventions known to inhibit the \(\text{Ca}^{2+}\)-activated component of I_{\text{Ca}^{2+}}^-.

Using a voltage-clamp technique, Tseng and Hoffman recently demonstrated two components of I_{\text{Ca}^{2+}}^- with widely different kinetics and pharmacological sensitivities in isolated canine ventricular myocytes that displayed a spike-and-dome morphology. The presence of two components of I_{\text{Ca}^{2+}}^- with brief and long reactivation and inactivation time constants has now been described in a large variety of cardiac tissues and cells (see Reference 1).

The present study suggests that I_{\text{Ca}^{2+}}^- in M cells is also comprised of two components with pharmacological sensitivities similar to those seen in EPI. The data presented in Figures 8–10 suggest that reactivation of I_{\text{Ca}^{2+}}^- in M cells is characterized by two components with fast and slow time constants. Both time constants, however, are slower than those encountered in EPI.

**Action Potential Duration**

APD recorded in M cells was generally longer than that recorded in EPI or ENDO (Table 1, Figures 5–8). Our findings are consistent with those of Solberg and coworkers, who encountered prolonged action potentials in the deep myocardial layers of transmural preparations obtained in the region of the papillary muscles (canine right and left ventricles). Action potentials with unusually prolonged APDs at slow rates have also been described in single myocytes dissociated from transmural preparations of canine, rat, and guinea pig ventricles.

In contrast, Van Dam and Durrer, using transmural measurements of refractoriness, reported that recovery of excitability across the free wall of the canine ventricle in vivo was somewhat erratic and generally more “advanced” in the midmyocardial layers. Using
similar techniques, Abildskov showed an EPI to ENDO recovery sequence. Although not highlighted, Figure 4 of Reference 28 indicates a distinctly longer refractory period in deep subepicardium.

**Rate and Time Dependence of Action Potential Duration**

It is well known that a change in the stimulation rate can alter APD in cardiac tissues. Acceleration-induced abbreviation of APD can be attributed to 1) incomplete recovery of ionic currents between one action potential and the next, 2) augmentation of electrogenic pump currents, and 3) changes in the activity of ions in the intracellular and extracellular compartments. The first two are primarily invoked to explain changes that occur after isolated premature beats or during the first few beats after an abrupt change of frequency, whereas the third accounts for the slower and more gradual changes in APD that develop after a change in rate.

Our data indicate that the rate dependence of APD is very much more accentuated in M cells than in EPI, ENDO, or deep subendocardium under both steady-state (Figures 5–7) and non–steady-state (Figure 8A) conditions. This characteristic feature is observed in both ventricles but is considerably more conspicuous in the left ventricle (compare Figures 7A and 7B). In contrast, the spike-and-dome morphology of EPI and M cells is generally more accentuated in the right ventricle (authors’ unpublished observations).

The pronounced rate dependence of APD exhibited by M cells is similar to that of Purkinje fibers or transitional cells and cannot be readily explained by rate-dependent changes in the early phases of the action potential, as in the case of EPI versus ENDO. Restitution of APD in M cells is likewise very similar to that observed in Purkinje fibers.

**Ionic Currents**

Voltage- and patch-clamp studies of single myocytes isolated from the M region will no doubt be necessary to define the ionic currents that participate in the generation of the M cell response and that distinguish it from those of other cells forming the ventricular myocardium. The relative contribution of some ionic currents, however, can be inferred from the available action potential data. A prominent contribution of \( I_{Na} \) to the M cell transmembrane response has already been discussed. A \( V_{max} \) value in M cells nearly twice that found in EPI or ENDO suggests that the intensity of the sodium current during the action potential upstroke may be considerably greater in these cells. The pronounced rate- and time-dependence of APD in M cells suggest that the currents underlying repolarization in these cells are also different from those in EPI or ENDO but perhaps similar to those in Purkinje fibers. The delayed rectifier current, although it contributes to

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**Figure 10.** Recovery of the available transient current as approximated by the phase 1 “difference voltage” produced by exposure of the preparation to 5 mM 4-aminopyridine (4-AP) (top inset). This is the same preparation as in Figure 9. Phase 1 amplitude (top inset) produced by 4-AP in premature beats introduced progressively later in the cycle is plotted on a logarithmic scale as a fraction of the maximum phase 1 amplitude (effect of 4-AP on the basic beats, basic cycle length of 2,000 msec). These values are plotted as a function of the diastolic interval, from full repolarization of the basic beat (S1) to the upstroke of the premature beat (S2). Bottom inset: Method used to derive time constant \( \tau \) (semilog plot). Curve is well fit by two exponential processes with time constants comparable to those obtained using the method shown in Figure 9A. All curves were fitted by eye.
repolarization in both ventricular and Purkinje myocytes, is thought to be more critical for repolarization in Purkinje cells. That M cells share this distinction is supported by the similarity of the rate dependence of APD in the two tissue types as well as by our recent finding that, like Purkinje but unlike ventricular EPI or ENDO, M cells develop early afterdepolarizations and early afterdepolarization--induced triggered activity after exposure to a variety of potassium channel blockers including quinidine, clofilium, cesium, 4-AP, and amiloride.38,39

**Physiological and Clinical Implications**

Our results provide further support for the existence of a marked heterogeneity of membrane properties and electrophysiological characteristics among cells spanning the ventricular wall of the canine heart. The presence of a deep subepicardial layer of cells displaying electrophysiological properties of both muscle and conductive tissues should contribute to our understanding of a number of basic electrophysiological and electrocardiographic phenomena.

**Specialized Subepicardial Conduction Pathway?**

The higher \(V_{\text{max}}\) exhibited by M cells begs the question as to whether these cells are part of a conduction system serving to facilitate impulse propagation in the deep subepicardial layers of the heart. Although a number of factors influence conduction in cardiac tissues, based on \(V_{\text{max}}\) values alone, M cells would be expected to conduct impulses at velocities intermediate between those of Purkinje fibers (1.0–4.0 m/sec) and ventricular muscle (0.4–0.6 m/sec). The longer and thinner appearance of these cells when compared with common ventricular myocytes is consistent with a specialized conduction function.11 The juxtaposition of layers of tissue with different conduction properties would be expected to contribute prominently to the anisotropic properties of the three-dimensional ventricular matrix.

**Supernormality**

The presence of a prominent \(I_n\) with slow reactivation kinetics has been shown to give rise to a phase of “excess overshoot” in canine ventricular EPI. During this period, the amplitudes of phases 0 and 1 of the action potentials of early premature beats are larger than those of responses elicited later in diastole.1 Because of this unique restitution characteristic, it is possible for premature beats to conduct under conditions in which basic beats are blocked (supernormal conduction). The possibility of supernormal conduction in ventricular muscle has long been a matter of debate. In vivo studies40,41 using extracellular stimulating electrodes (in many cases applied to the ventricular EPI) have demonstrated a supernormal period, whereas studies of ventricular muscle tissues (ENDO) in vitro have failed to uncover a period of supernormal conduction or excitability.42 In a recent study, we demonstrated supernormal conduction in EPI strips of tissue whose central segment was depressed by superfusion with an “ion-free” isotonic sucrose solution. Under conditions in which conduction of the basic beats was totally blocked, early premature beats were shown to successfully conduct across the inexcitable zone (sucrose gap). Successful conduction of the premature beats was due to the greater amplitudes of the early phases of the EPI action potential, which provide for a much greater source current in early versus late diastole. These characteristics of EPI are shared by M cells (see Figures 2, 8, 9, and 10), suggesting that these cells may contribute to the manifestation of a supernormal phase of conduction in ventricular myocardium.

**Intramural Reentry**

The much steeper APD–rate relation exhibited by cells in the M region relative to other parts of the ventricular myocardium gives rise to a progressively and more prominent dispersion of repolarization and refractoriness within the ventricular wall as stimulation rate is slowed. A deep subepicardial or midmyocardial “wall” of refractoriness or “arc of block” would be expected to develop at slow rates, setting the stage for a variety of reentrant arrhythmias, intramural reentry in particular. In preliminary studies we have also observed that cells in the M region are more resistant than EPI or ENDO cells to K+ depolarization, suggesting that M cells may be able to better tolerate ischemic conditions and thus may contribute to the development of intramural reentry and other arrhythmias.

Intramural reentry has recently been identified as the principal mechanism underlying the initiation and maintenance of ventricular tachycardia leading to ventricular fibrillation during ischemia.44–47 Also of interest are recent preliminary reports by Patter-son and Sherlag46,49 showing pause- and bradycardia-dependent facilitation of midmyocardial reentry after 15–30 minutes of ischemia (phase IB arrhythmias; coronary artery occlusion in a canine heart in vivo). The bradycardia-induced arrhythmias were accompanied by the development of prominent midmyocardial activation delays and continuous diastolic midmyocardial electrical activity.

**Triggered Activity**

In a corollary study,38 we have found that outward current blocking agents, such as quinidine, cesium, and 4-AP, produce early afterdepolarizations and early afterdepolarization–induced triggered activity in M cells but not in ENDO or EPI. Similar findings were noted with amiloride and clofilium.39 Other studies have demonstrated that M cells, but not EPI or ENDO cells, exhibit delayed afterdepolarizations and delayed afterdepolarization–induced triggered activity in response to digitalis, catecholamines, and hypercalcemic conditions.39

The capability of these deep subepicardial cells to generate triggered activity may explain a variety of electrocardiographic manifestations, including reports of pacemakerlike activity or ectopic activity in
the form of bigeminy arising from localized subepicardial sites.50,51

Long QT Intervals, U Waves, and Torsade de Pointes

Our findings suggest that the presence of M cells in the deep subepicardium may contribute to a number of electrocardiographic manifestations, including long QT intervals, U waves, and drug-induced torsade de pointes.52–56

Electrocardiographic U waves and long QT intervals are generally seen under bradycardic conditions, and their manifestation is frequently enhanced by drugs known to prolong APD and/or induce early afterdepolarizations in the specialized conduction system of the ventricle. A number of hypotheses have been advanced to explain these electrocardiographic phenomena. Prominent among these is the hypothesis that both manifestations are due to late repolarization of the Purkinje system and/or the development of early or delayed afterdepolarizations in the Purkinje system.57,58 A criticism of this hypothesis has been that the conduction system of the ventricle does not have sufficient mass to generate a distinct U wave on a surface electrocardiogram. The presence of another population of cells in the deep subepicardium with similar behavior may provide the critical mass.

The development of early afterdepolarizations and marked action potential prolongation in M cells in response to drugs such as quinidine also provides further insight into our understanding of long QT intervals and atypical ventricular tachyarrhythmias like torsade de pointes that develop under bradycardic and hypokalemic conditions in patients undergoing quinidine therapy (see Reference 59 for further references).

Finally, the presence of a prominent notch in EPI and M cell action potentials, but not those of ENDO, would be expected to produce a ventricular gradient that may contribute to the manifestation of a J wave or Osborne wave in the electrocardiogram.43

Conclusion

Our study provides evidence in support of the existence of a unique population of cells in the deep subepicardial layers of the canine ventricle possessing electrophysiological and pharmacological properties intermediate between those of conducting and myocardial cells of the heart. It is hoped that these findings will prompt further electrophysiological and morphological studies into the nature, function, ionic basis, and pharmacological responsiveness of these cells, ultimately advancing our understanding of mechanisms of arrhythmia and conduction disturbances and providing new insight for pharmacological as well as invasive (e.g., pacemaker implantation and surgical techniques) approaches to therapy.

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