Characteristics and Distribution of M Cells in Arterially Perfused Canine Left Ventricular Wedge Preparations

Gan-Xin Yan, MD, PhD; Wataru Shimizu, MD, PhD; Charles Antzelevitch, PhD

Background—Much of the characterization of the M cell to date has been accomplished using isolated tissues and cells. This study uses an arterially perfused wedge preparation to examine the characteristics and distribution of M cells within the anterior wall of the canine left ventricle under more physiological conditions.

Methods and Results—Floating microelectrodes were used to record transmembrane action potentials simultaneously from epicardial, M region, and endocardial or subendocardial Purkinje sites in isolated arterially perfused canine left ventricular wedge preparations. A transmural ECG was recorded concurrently. M cells with the longest action potentials were found in the deep subendocardium in wedge preparations isolated from the anterior wall of the left ventricle. Fairly smooth transitions in action potential duration (APD) were observed except in the region between epicardium and deep subepicardium. Tissue resistivity increased 2.8-fold in this region and much more modestly in the deep subendocardium. Dispersion of APD across the left ventricular wall averaged 51 ± 19 and 64 ± 25 ms at basic cycle lengths of 1000 and 2000 ms, respectively, whereas transmural dispersion of repolarization time was smaller (34 ± 18 and 45 ± 25 ms), owing to the endocardial to epicardial activation sequence.

Conclusions—We conclude that the qualitative differences between the 3 ventricular cell types previously described in isolated tissues and cells are maintained in intact canine left ventricular wall preparations in which the myocardial cells are electrically well coupled. As anticipated, differences in APD are quantitatively smaller because of electrotonic interactions among the 3 cell types. Our data indicate that transmural dispersion of repolarization is the result of intrinsic differences in APD of cells spanning the ventricular wall as well as a heterogeneous distribution of tissue resistivity across the wall. (Circulation. 1998;98:1921-1927.)

Key Words: M cells ■ electrophysiology ■ heterogeneity ■ hypokalemia ■ action potentials

M cells are distinguished by the ability of their action potentials to prolong disproportionately compared with those of other ventricular myocardial cells in response to a slowing of rate and/or to agents that prolong action potential duration (APD).1,2 M cells have been described in the ventricles of human,3,4 dog,5,13 guinea pig,6 and rabbit7 hearts. The only mammalian species in which they were thought to be lacking is the pig,8 but their absence in this study may be related to maturity rather than species differences.9 Histologically, M cells appear similar to epicardial and endocardial cells. Electrophysiologically and pharmacologically, they are more similar to Purkinje cells, although important differences have been described.

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Much of the characterization of the M cell to date has been accomplished using isolated tissues and cells studied under less than physiological conditions (for review see references 10 through 12). Although these methodologies have permitted a critical assessment of the intrinsic properties of this unique cell type, they have provided little information about the interaction of M cells with their neighbors or their characteristics within the functional syncytium that comprises the ventricular myocardium.

To observe M cells in an environment in which they are electrically well-coupled to neighboring cells, we developed an arterially perfused preparation consisting of a wedge of canine left ventricle in which we are able to simultaneously record transmembrane activity from epicardial, M, and endocardial or subendocardial Purkinje sites along the transmural surface of the ventricular wall using floating glass microelectrodes.13 In this study, we use the wedge preparation to define the distribution of M cells across the anterior ventricular wall of the canine left ventricle and to study their action potential characteristics under more physiological conditions.

Methods

Arterially Perfused Wedge of Canine Ventricle

Mongrel male dogs weighing 20 to 25 kg were anticoagulated with heparin and anesthetized with pentobarbital (30 to 35 mg/kg IV). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4°C) or room-temperature Tyrode’s solution containing 8.5 mmol/L [K⁺]. Transmural wedges with dimensions of approximately 2 × 1.5 × 1 cm to 3 × 2 × 1.5 cm were dissected from the anterior wall of the left ventricle. The

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tissue was cannulated via a small (diameter = 100 μm) native branch of left descending coronary artery and perfused with cardioplegic solution. The total time from excision of the heart to cannulation and perfusion of the artery was <4 minutes in all experiments. Unperfused tissue, readily identified by its maintained red appearance (erythrocytes not washed away), was carefully removed using a razor blade. The preparation was then placed in a small tissue bath and arterially perfused with Tyrode’s solution of the following composition (mM/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). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Inc) and maintained between 40 and 50 mm Hg by adjustment of the perfusion flow rate. The preparations remained immersed in the arterial perfusate, which was allowed to rise to a level 2 to 3 mm above the tissue surface when possible. It was often difficult to maintain the floating microelectrode impalements with the bath solution above the height of the tissue. In many experiments, the level of the solution had to be lowered to just below the top of the wedge. Temperature gradients could be observed in the wedge along the superior-inferior axis under these conditions. Large temperature gradients (cooler temperatures on top) typically resulted in an M-cell action potential (AP) that lasted the end of the T wave. This problem was greatly minimized and, in most cases, eliminated by placing a series of coverslips at the top of the tissue chamber so as to insulate it from the much cooler room air. Temperature gradients were quantified using a fine tip temperature sensor. Experiments in which the temperature gradients were not adequately controlled (ΔT > 1.5°C) were excluded from the study. In a few cases, we normalized the transmembrane signals to the peak amplitude of the AP when the amplitude of these signals was reduced due to less than optimal impalement; the temporal parameters of the scaled AP (eg, APD90) were identical to those of the well-impaled response.

The ST-segment depression initially observed normalized within 10 to 15 minutes of start of perfusion. In preparations in which an ST-segment deviation of >10% of the amplitude of the transmural QRS voltage persisted beyond the initial 15-minute period, the preparation was discarded. With this criteria, we were able to select wedge preparations that remained electrically stable for a period of at least 4 hours.

**Recordings of Transmembrane Action Potentials**

The wedge preparations were allowed to equilibrate in the tissue bath until electrically stable, usually 1 hour. The preparations were stimulated using bipolar silver electrodes insulated except at the tips and applied to the endocardial surface.

Transmembrane action potentials were simultaneously recorded from the epicardial, M, and endocardial or subendocardial Purkinje sites using 3 to 4 separate intracellular floating microelectrodes (DC resistance, 10 to 20 MΩ) filled with 2.7 mol/L KCl and connected to a high-input impedance amplifier (Figure 1). Transmural distances were measured using an eyepiece micrometer mounted in a binocular microscope. Recordings were designated to 1 of 10 or 11 transmural bins.

All amplified signals were digitized, stored on magnetic media and WORM-CD, and analyzed using Spike 2 (Cambridge Electronic Design). All APD measurements are reported at 90% repolarization (APD90).

**Measurement of Tissue Resistivity Within the Left Ventricular Wall**

Mathematical simulations have suggested that the morphology of the T-U complex in the ECG may be greatly influenced by the distribution of tissue resistance within the ventricular wall. In the present study, we provide a direct test of this hypothesis. We assess the distribution of resistance across the ventricular wall by measuring total tissue resistivity (Rt), which reflects extracellular and intracellular resistivity in parallel.

![Figure 1. Schematic of an arterially perfused canine left ventricular wedge preparation.](image)

The perfused wedge was suspended in a water-saturated atmosphere to obtain a constant ratio of extracellular to intracellular resistance. A subthreshold current (I) was delivered across the wall via 2 silver plates placed at the endocardial and epicardial sides of the wedge. The silver plates were 1 × 1 cm², similar in size to the surfaces of the wedge to ensure that the current flow was uniform across the wedge. The results of tissue-resistivity measurements were similar, independent of the polarity of the applied current. The specific tissue resistance (r t) across the transmural wall was mapped using 2 extracellular electrodes spaced ~1 mm (Ax) apart and advanced across the wall in increments of ~1 mm:

\[
r_{\text{trans}}(\Omega/cm) = V_o(x)/I\Delta x
\]

where \(V_o(x)\) is the amplitude of the extracellular bipolar electrogram recorded during the flow of the subthreshold current between the 2 extracellular electrodes. Rt is calculated on the basis of the values of r t and the cross-sectional area (S) of the preparation as follows:

\[
R_t(\Omega/cm) = r_t \times S
\]

When the extracellular subthreshold current I is injected at the epicardial or endocardial site, part of it flows through the cell membrane into the intracellular compartment. Beyond a distance of 3 space constants (A) from the current injection site, extracellular and intracellular currents become constant and flow parallel through the remainder of the preparation. At sites <3A from the current source, the measured r t will be overestimated. Corrected values of r t can be calculated using the following approach: Extracellular voltage difference \(V_e(x)\) produced by injected current I can be estimated from the cable equation as follows (see equation 5 in reference 16):

\[
V_e(x) = -\int_0^x \left[ \frac{1}{r_t + r_i} \left( \frac{r_s}{r_i + r_s} \right) \left( 1 - e^{-x/r_i} \right) \right] r_e dx = -\frac{1}{r_t} \int_0^x \left( 1 - e^{-x/r_i} \right) dx
\]

where

\[
r_e = \frac{r_i r_s}{r_i + r_s}
\]

Resistance r t is then calculated from the experimentally measured value \(r_{\text{trans}}\) using the following correction:
Purkinje system, 3 myocardial cell types have been described in addition to the specialized conducting cells of the His-
tal. Statistical analysis of the data was performed using Student’s t
test for paired data or 1-way ANOVA coupled with Scheffe’s test. All
results are expressed as mean±SD unless otherwise indicated.

Results
In addition to the specialized conducting cells of the His-
Purkinje system, 3 myocardial cell types have been described
in the ventricles of mammalian hearts. The presence of a
more prominent transient outward current (Ito) contributes to
the appearance of a prominent notch in the early phase of
epicardial and midmyocardial action potentials, whereas a
smaller delayed rectifier current (IKs) and larger late sodium
current (INa) contribute to the appearance of longer action
potentials in midmyocardial M cells. Transmural dispersion of
APD is illustrated in Figure 2. The 3 top action potentials were simultaneously
recorded from the epicardial, endocardial, and M regions of a
wedge preparation stimulated from the endocardial surface at
a basic cycle length (BCL) of 1000 (Figure 2A) and 4000 (Figure 2B) ms. The lower traces depict action potentials recorded from a subendocardial Purkinje in the same prepa-
ration. The Purkinje response is longer than that of the M cell
and can also be distinguished from that of the M cell by the
absence of a distinct notch. Among the myocardial cells, the
action potential of the epicardial cell is the briefest, whereas
that of the M cell is the longest; thus the difference in APD
between the epicardium and M region defines the maximal
transmural dispersion of APD (Table).

\[
\frac{r_e}{r_i} = \frac{1}{1 + \frac{r_e}{r_i} \int_0^{\Delta x} e^{-x/\lambda} \, dx} \quad \text{or} \quad \frac{r_e}{r_i} = \frac{1}{1 + \frac{r_e}{r_i} e^{-\Delta x/\lambda}}
\]

To arrive at this correction factor, we determined the ratio of
extracellular to intracellular resistance (\(r_e/r_i\)) in canine right ventricular papillary muscles by applying cable analysis.\(^6,\)\(^7\) The ratio obtained was \(r_e/r_i = 0.66±0.13\) \((n=4)\) when the canine papillary muscle preparation was perfused with 
Tyrode’s solution. Based on the assumptions that the ratio of
extracellular to intracellular resistance of left ventricular wall
is similar to that of the papillary muscle and that \(\lambda\) is
\(\approx 0.5\) mm, \(R_e\) in the first millimeter of epicardium and
endocardium was calculated by substituting these values in
equation 4:

\[
R_e = r_{\text{mean}} \left(1 + 0.66 \cdot e^{-10.5/0.5}\right) \times 1000 \times 0.778 \times S
\]

Histology of Transmural Slice
Left ventricular tissues were isolated from adult mongrel dog hearts,
fixed in 10% buffered formalin for 24 hours, and embedded in
paraffin. Several transverse sections of 5-μm thickness were cut and
stained with hematoxylin and eosin according to standard proce-
dures. Briefly, sections were deparaffinized in Hemo-De (Fisher
Scientific) for 10 minutes and then rehydrated in graded ethanols
ranging from 50% to 100%, and
rehydrated in graded ethanols ranging from 100% to 50%. The sections were then rinsed with water
and stained with Mayer’s hematoxylin (Sigma Chemical Co) for 5
minutes. The sections were rinsed in water, differentiated in acid
alcohol for 1 minute, and then counterstained with eosin Y for 5
minutes. The sections were then rinsed in water for 2 minutes,
dehydrated in graded ethanols ranging from 50% to 100%, and
mounted using Permount (Fisher Scientific). Tissue sections were
analyzed and photographs were obtained using Polaroid Microcam
camera attached to a Leica microscope.

Statistics
Statistical analysis of the data was performed using Student’s t
test for paired data or 1-way ANOVA coupled with Scheffe’s test. All
results are expressed as mean±SD unless otherwise indicated.

<table>
<thead>
<tr>
<th>APD90 (ms)</th>
<th>Transmural Dispersion of APD90 (ms)</th>
<th>Transmural Dispersion of Repolarization Time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCL, 1000</td>
<td>BCL, 2000</td>
</tr>
<tr>
<td>Epicardium</td>
<td>207±20</td>
<td>217±24</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M cell</td>
<td>260±21</td>
<td>281±25</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium</td>
<td>249±18</td>
<td>266±21</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subendocardial Purkinje fibers</td>
<td>299±17</td>
<td>326±19</td>
</tr>
<tr>
<td>(n=14)</td>
<td></td>
<td></td>
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</tbody>
</table>

BCL, basic cycle length. Values are mean±SD (in ms).
Transmural dispersion of APD in the wedge ranged between 32 and 85 ms and averaged 64 ± 25 ms at a BCL of 2000 ms (Table). This contrasts with an average dispersion of APD of 105 ± 45 ms in isolated tissue slices and 170 ± 51 ms in myocytes. These differences are anticipated, because 3-dimensional electrical coupling of cells in the wedge permits electrotonic influences that abbreviate the APD of the M cell below its intrinsic value and prolong the APD of epicardial and endocardial cells above their intrinsic durations.

To assess the distribution of cell types across the anterior free wall, we used a roving floating microelectrode in conjunction with 2 stationary microelectrodes at epicardial and endocardial sites to map the distribution of action potential characteristics across the wall in arterially perfused wedge preparations isolated from canine ventricles. Figure 3 illustrates the results obtained from one such preparation. APD at 90% repolarization (APD₉₀) is briefest at the epicardial border, increases sharply in the deep subepicardium and then more gradually, peaking within the subendocardium. Average transmural conduction time was 29.3 ± 1 ms (at BCL=1000 ms), and the average thickness of the wedge preparations was 12.9 ± 1.5 mm; the average conduction velocity of 44 cm/s is comparable to that recorded in vivo. The dispersion of repolarization time (RT = APD₉₀ + conduction time) across the wall is less than the transmural dispersion of APD₉₀ because of the endocardial-to-epicardial activation sequence.

Figure 4 summarizes the results of 15 experiments. APD₉₀ is significantly shorter in epicardial cells than in either M cells (deep subendocardium) or endocardial cells. A sharp transition of APD is observed between epicardium and deep subepicardium, with more gradual transitions appearing throughout the remainder of the wall. The data clearly indicate that M cells with the longest action potentials are located in the deep subendocardium in the anterior wall of the canine left ventricle.

Figure 5 illustrates the results obtained from one such preparation. APD at 90% repolarization (APD₉₀) is briefest at the epicardial border, increases sharply in the deep subepicardium and then more gradually, peaking within the subendocardium. Average transmural conduction time was 29.3 ± 1 ms (at BCL=1000 ms), and the average thickness of the wedge preparations was 12.9 ± 1.5 mm; the average conduction velocity of 44 cm/s is comparable to that recorded in vivo. The dispersion of repolarization time (RT = APD₉₀ + conduction time) across the wall is less than the transmural dispersion of APD₉₀ because of the endocardial-to-epicardial activation sequence.

The sharp transitions of APD₉₀ observed in Figures 3 and 4, suggest the presence of a resistive barrier between epicardium and deep subepicardium. To test this hypothesis, we measured tissue resistivity across the left ventricular wall. The distribution of Rₑ, a reflection of intracellular and extracellular resistivity in parallel, measured across the left ventricular wall is illustrated in Figure 5 (n=5). Rₑ values in the region between epicardium and deep subepicardium (0% to 20% of transmural thickness) average 410 ± 55 Ω cm, 2.8-fold greater than that in midmyocardium (average resistivity of the 20% to 80% segment of the wall was 148 ± 11 Ω cm; P<0.01). The Rₑ between endocardium and deep subendocardium was observed to vary greatly, yielding a mean value of 203.4 ± 47.9 Ω cm, 1.4-fold greater than that in midmyocardium (P=0.3, Figure 5).

The dramatic increase in Rₑ near the epicardial border may be because of differences in either rₒ or rᵢ secondary to changes in the extracellular matrix or gap junctional resi-
tance, respectively. Figure 6 shows a representative example of the histology encountered in this part of the ventricular wall. The segment, consisting of the epicardial third of a transmural slice, shows a sharp transition of cell orientation in the region of high tissue resistivity. Similar sharp transitions in cell orientation in the deep subepicardium were observed in 8 of 8 preparations.

Previous studies have demonstrated major differences in the APD-rate relations of the 3 ventricular myocardial cell types. When studied in isolated tissues and myocytes, M cells display a steep rate dependence, whereas epicardial and endocardial cells show relatively little change in APD as a function of rate. The electrotonic currents that flow during normal electrical coupling of cells in a functional syncytium would be expected to blur these sharp distinctions in the intrinsic behavior of the 3 cell types. As a test of this hypothesis, we examined the rate dependence of action potential characteristics using 3 simultaneous floating microelectrode recordings obtained from the epicardial, endocardial, and M regions (Figure 7).

In support of the hypothesis, the APD$_{90}$ rate relation of the M cell was much less accentuated than previously reported in isolated tissues and cells, whereas the APD$_{90}$ rate relations of epicardium and endocardium were considerably more accentuated (Figure 7).

**Discussion**

The discovery of cells in the midmyocardial layers of ventricular myocardium with unique repolarization properties has prompted investigations into the distinctive electrophysiological, pharmacological, and pathophysiological characteristics of these cells. The chief attribute of the M cell is the ability of its action potential to prolong disproportionately compared with that of other myocardial cells in the ventricle in response to a slowing of rate and/or to agents that prolong APD.$^{1,2,5}$ The ionic basis for these features of the M cell includes the presence of a smaller slowly activating $I_{Ks}$ as well as a larger late $I_{Na}$. The present study examines the characteristics and distribution of M cells within the intact wall of the canine left ventricle using floating glass microelectrodes to simultaneously record transmembrane action potentials from the transmural sites along the ventricular wall of an arterially perfused wedge preparation.

Previous studies have validated the model showing that action potentials along the transmural surface recorded using floating microelectrodes yield APD values very similar to the activation recovery interval (ARI) values recorded using intramural electrodes placed in the subtending tissue.$^{22,23}$ The viability and electrical stability of the preparation over a 4-hour period was demonstrated as well.$^{14}$

**Transmural Distribution of APD**

Although M cells have been described in a variety of species, including humans, their precise location within the ventricular wall has been investigated in greatest detail in the LV of the canine heart. Whereas transitional cells are found throughout most of the wall in the canine LV, M cells displaying the longest action potentials (at slow rates) have been localized to the deep subepicardium to midmyocardium in the lateral wall,$^1$ deep subendocardium of the septum,$^{24}$ and throughout the wall in the region of the outflow tracts.$^{5,10}$ The present study demonstrates that in the anterior wall of the canine left ventricle, M cells with the longest action potentials are located in the deep subendocardium. The shift in the position of the M cells appears to correspond to the myocardial layers described in early studies by Streeter$^{25,26}$ and more recently by Lukenheimer and coworkers.$^{27}$
Transmural Distribution of Resistivity
Theoretically, the manifestation of electrical heterogeneity across the intact ventricular wall depends on (1) the extent to which intrinsic action potential characteristics of neighboring cells differ and (2) the extent to which they are electrically coupled in the syncytium.29 Where coupling resistance is very low, intrinsic differences in the APD of the different cell types will be obscured over relatively short distances but may be perceptible over greater distances. As coupling resistance increases, so does the ability to manifest differences of APD at neighboring sites. The present study demonstrates a heterogeneous distribution of resistance across the wall of the canine left ventricle (Figure 5). The sharp increase in R, between the M region and epicardium can account for the sharp increase in APD within this region of the wall. This resistive barrier is probably still more important in the lateral free wall of the left ventricle where the longest M cells are found in the deep subepicardial to midmyocardial layers.1

Despite the relatively large increase in tissue resistivity in the deep subepicardium, conduction in this region slowed only slightly, consistent with cable theory predictions. The basis for the sharp rise in tissue resistivity is not known, an abrupt shift in the orientation of the myocardial cells in this part of the wall may contribute (Figure 6). A similar shift in cell orientation has been documented in the deep subepicardium of the human left ventricle, where prolonged M-cell action potentials are first encountered.1

Transmural Dispersion of Repolarization
The degree of transmural dispersion of repolarization observed across the ventricular wall depends on the methods used to record this parameter. In the absence of drugs, but at slow rates, tissues or myocytes isolated from the M region display APDs as much as a couple of hundred ms longer than those recorded from endocardium or epicardium.1,20,29,38 When recorded from the intact left ventricular wall of arterially perfused wedge preparations, where the 3 cell types are electrotonically well coupled, the dispersion of repolarization is reduced to 51±19 ms (at BCL=1000 ms) because of the electrotonic forces that act to abbreviate the M-cell action potential below its intrinsic APD and to prolong epicardial and endocardial cell action potentials beyond their intrinsic APDs (Figures 2 through 6, Table).34 A similar dispersion of repolarization (30 to 40 ms at BCL=1400 to 1500 ms, anesthetized dogs) is observed in the canine heart in vivo when monophasic action potentials (MAP) or unipolar recordings are used to estimate the MAP duration (MAPD) or ARI.31,32 ARI measurements provide a reasonable approximation of local repolarization time under all but extreme conditions (greatly exaggerated repolarization gradients).22 In the accompanying article,23 we will present evidence in support of the hypothesis that the transmural dispersion of repolarization contributes importantly to inscription of the electrocardiographic T wave.

The transmural dispersion of repolarization observed under control conditions in the canine heart in vivo and in the wedge preparations increases dramatically in the presence of agents with Class III actions such as d-sotalol, erythromycin, and anthopleurin A because of the preferential action of these agents to prolong the APD of the M cell.14,22,23 Transmural repolarization gradients as large as 150 ms can be observed under these conditions. As will be illustrated in the accompanying paper, this amplification of transmural dispersion of repolarization is due to a preferential action of Class III agents to prolong the APD of the M cell.23

Another factor that can influence the degree to which transmural heterogeneity can be discerned is the method used to approximate repolarization time. Transmembrane action potential recordings provide the most direct and accurate measurement of local repolarization. ARI obtained using unipolar extracellular electrodes and measurement of local refractoriness provide a fairly good approximation.2,32–34 Whereas unipolar electrograms provide information that can be interpreted on the basis of biophysical theory,34,35 bipolar electrograms, although providing a sharper activation complex, generate a repolarization complex that is not as readily interpretable because it represents the difference in the activity of 2 sites. As a result, it is difficult to make a distinction between ARIs at the 2 sites; and when differences exist, they are likely to be obscured with bipolar recording techniques.

Recent preliminary reports point to the anesthesia employed as another confounding factor in the determination of transmural dispersion of repolarization in in vivo studies.36 The dispersion of repolarization measured across the anterior left ventricular wall (transmural MAP recordings) is considerably smaller when sodium pentobarbital is used for general anesthesia than when halothane is used. This difference, seen under baseline conditions, is still more exaggerated when an I$_{Kr}$ blocker such as d-sotalol is infused.31,37 d-Sotalol produces a dramatic increase in transmural dispersion of repolarization when halothane is used for anesthesia but not when sodium pentobarbital is used.31 An abbreviation of the M-cell action potential but prolongation of the epicardial and endocardial APDs underlie these effect of pentobarbital to reduce transmural dispersion.

These and other differences in method may account for the failure of some studies to discern significant repolarization gradients across the canine left ventricular wall in vivo38,39 and the ability of others to demonstrate them reproducibly.31,32,40 A relatively small transmural dispersion of repolarization has been reported (at slow rates) in in vivo studies that have used pentobarbital for anesthesia5,31,38 versus studies that have used other agents including isoflurane32 and halothane.31 Although significant transmural repolarization gradients are observed with halothane and isoflurane, these gradients measured in vivo are slightly smaller than in the isolated wedge (following washout of anesthetic). Further studies are needed to determine whether this difference is because of an effect of the halothane (and also isoflurane) to reduce transmural dispersion of repolarization, although to a lesser extent than sodium pentobarbital, thus leading to underestimation of the transmural gradients present in the awake state. A wider field of view of the unipolar electrodes relative to transmembrane electrodes may also result in underestimation of transmural gradients. Finally, small differences in intercellular resistance between wedge and in vivo conditions could,
in theory, contribute to differences in the degree to which transmural dispersion of repolarization is observed.

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