Repolarization Gradients in the Canine Left Ventricle Before and After Induction of Short-Term Cardiac Memory

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Background—Questions remain about the contributions of transmural versus apicobasal repolarization gradients to the configuration of the T wave in control settings and after the induction of short-term cardiac memory.

Methods and Results—Short-term cardiac memory is seen as T-wave changes induced by altered ventricular activation that persists after restoration of sinus rhythm. We studied cardiac memory in anesthetized, open-chest dogs paced from the ventricle for 2 hours. Unipolar electrograms were recorded from as many as 98 epicardial and 144 intramural sites, and activation times and activation-recovery intervals (ARIs) were measured. In separate experiments, epicardial monophasic action potentials were recorded. We found no appreciable left ventricular intramural gradients in repolarization times (activation time + ARI) in either control conditions or after the induction of memory. In controls, there was a left ventricular apicobasal gradient, with the shortest repolarization times in anterobasal regions and longest repolarization times posteroapically. After induction of memory, repolarization times shortened uniformly throughout the ventricular wall. Monophasic action potential duration at 90% repolarization decreased by \( \approx 10 \) ms after induction of memory.

Conclusions—In the intact canine left ventricle at physiological rates, there is no transmural gradient in repolarization. Apicobasal gradients in repolarization time, with shortest repolarization times in anterobasal areas and longest repolarization times in posteroapical regions, are important in the genesis of the T wave. Repolarization times and monophasic action potentials at the 90% repolarization level shorten after the induction of memory. The deeper T wave in the ECG after induction of memory may be explained by the more rapid phase 3 of the action potential. (Circulation. 2005;112:1711-1718.)

Key Words: action potentials ■ electrocardiography ■ electrophysiology ■ mapping ■ ventricles

Cardiac memory is defined as an altered T-wave morphology induced by ventricular pacing or arrhythmia that persists after resumption of sinus rhythm. The T wave may reflect both transmural and apicobasal gradients in repolarization, but most authors consider the repolarization gradient across the ventricular wall as the most important determinant of the T wave. In both long-term memory (weeks of ventricular pacing) and in models of short-term memory (\( \leq 2 \) hours of pacing), alterations in the transmural gradient for repolarization have been reported, the former based on action potential (AP) recordings from isolated tissue. However, no extensive mapping of the epicardium, endocardium, and intramural myocardium has been performed before and after the induction of cardiac memory. Therefore, we recorded unipolar electrograms simultaneously from as many as 98 epicardial and 144 intramural electrodes in canine hearts. Activation times and activation-recovery intervals (ARIs) were measured from these electrograms. In addition, epicardial monophasic APs were recorded. To document cardiac memory, electrocardiograms and frontal-plane vector cardiograms were recorded as well.

We found no appreciable intramural gradients of repolarization time (activation time + ARI) in the control situation and after induction of cardiac memory. In contrast, during control experiments, there was an apicobasal gradient, with the shortest repolarization times in anterior basal regions and longest repolarization times in posteroapical regions. After induction of memory, repolarization times shortened throughout the ventricular wall, with epicardial monophasic APs decreasing by \( \approx 10 \) ms at the 90% repolarization level. The deeper T wave in the ECG after induction of memory may be explained by the more rapid phase 3 of the AP.
Methods

The protocols were approved by the Columbia University Animal Care and Use Committee. Male mongrel dogs 1 to 3 years old and weighing 22 to 26 kg were anesthetized with thiopental 17 mg/kg IV, intubated, and ventilated with isoflurane (1.5% to 3.0%) and oxygen (2 L/min). The left femoral artery was catheterized to monitor blood pressure. A heating pad was used to maintain body temperature. The heart was suspended in a pericardial cradle via a thoracotomy at the fifth left intercostal space. Temperature was monitored regularly at the anterior epicardial surface and deep in the pericardial cradle. The maximum difference in temperature between these 2 sites, as well as at the same site during the course of any experiment, was ≤0.5°C.

Platinum bipolar electrodes sewn epicardially to the left atrial appendage and the inferolateral left ventricular wall were used for pacing. A 6-lead ECG was continuously recorded by the IOX acquisition system (EMKA Technologies).

In 4 dogs, epicardial sock electrodes (98 electrode terminals organized in 7 strips each, harboring 2 rows of 7 electrodes separated by 1.5 cm) were sutured onto the surface of right and left ventricles. In addition, 42 plunge electrodes (0.5-mm diameter) with terminals at distances of 4 mm were inserted. Twenty-four electrodes were inserted into 9 segments of the free wall of the left ventricle with intramural recordings at the subepicardium and at depths of 4, 8, and 12 mm, yielding a total of 96 recordings. Twelve electrodes were inserted into 6 segments of the interventricular septum, with intramural recordings on the left and right endocardial surfaces and in the midseptum, resulting in 36 recordings. Finally, 6 electrodes were inserted into 3 segments of the free wall of the right ventricle, at the subepicardium, and at a depth of 2 mm, yielding 12 recordings. Unipolar electrograms from as many as 98 epicardial electrodes and as many as 144 intramural electrodes and 5 surface ECG signals were simultaneously recorded with a personal computer–based data acquisition system. The reference signal was derived from a virtual ground electrode connected to the mediastinum. Selected episodes of data could be stored on the hard disk of the computer. Sampling interval was 0.5 ms. Analysis of the signals was done offline with a custom-made data analysis program.

The previously described protocol for inducing short-term memory was as follows. After an equilibration period of atrial pacing at a cycle length of 500 ms, the ventricles were paced for 2 hours at a cycle length of 400 ms at an apicolateral site. After 2 hours of ventricular pacing, atrial pacing was resumed at a cycle length of 500 ms to evaluate the magnitude of cardiac memory (4 minutes before shifting from ventricular to atrial pacing, the cycle length was increased to 500 ms to avoid the influence of rate when recording during atrial pacing). ECGs were recorded online, and frontal-plane vector images were plotted with Dr Vetter PC-EKG software (Dr Vetter), as previously described.

Although in this study no control experiments were performed, in previous studies, atrial pacing at the same rate and duration as those used in the ventricular pacing protocol produced no significant changes in the ECG and the vector cardiogram. Data reported here were collected during the first 5 minutes after returning to atrial pacing.

Activation times were measured as the interval between the beginning of the surface QRS complex and the time of maximum negative slope of the unipolar QRS complex; ARIs, which are well correlated with local transmembrane AP duration or refractory periods, were measured as the interval between the moment of activation and the maximum positive slope of the electrogram T wave.

Cardiac memory was quantified as a function of T-wave vector amplitude and angle changes and expressed as the distance between frontal-plane T-wave vector peaks recorded during atrial pacing in the control situation and at subsequent time points (T-wave vector displacement), as previously described.

In 4 separate experiments with the same pacing and recording protocols, monophasic APs were recorded from 12 epicardial sites...
by using the contact-electrode method described by Franz.16 The signals were amplified and filtered with a monophasic AP amplifier (EP Technology) and fed into the acquisition system.

All statistical analyses were performed as repeated-measures analyses with the 4 hearts as the experimental units. Not every measurement at every electrode and at every position was available before and after induction of memory. To solve the problem of missing values, we averaged the available measurements over needles and at certain positions. Thus, to study the influence of transmural position on repolarization time, we averaged all available measurements at the endocardial, 8-mm, 4-mm, and epicardial positions. The results (per dog, per transmural position, and per memory status) are shown as data points in Figure 4. The data points of Figures 3 and 6 were obtained in a similar fashion. The reported probability values are those of the test for a linear trend in the repeated (ie, within dogs) measurements.

**Results**

**Cardiac Memory and Transmural Repolarization**

Figure 1 shows the extremity ECG leads in a single experiment in control conditions and after 2 hours of ventricular pacing on returning to atrial pacing. Note the deepening of the T wave during the second period of atrial pacing. In this experiment, the T-wave vector displacement was 0.30 mV (not shown). The right panel shows an enlargement of ECG lead II to emphasize not only the deeper T wave but also the shortening of the QT interval after induction of memory.

In Figure 2, unipolar electrograms from 1 intramural needle electrode in the anterior free wall of the left ventricle are shown, at the epicardium, and at depths of 4, 8, and 12 mm (close to the endocardium [Endo]). Note the shortening of all QT intervals in memory.

| TABLE 1. Shortening of ARIs by Cardiac Memory in 4 Canine Hearts |
|------------------|------------------|------------------|
| Heart No. | ARI (Memory − Control), ms | SD, ms | SEM, ms | Sites With a Decrease in ARI |
| 1 | −2.4 | 5.8 | 0.7 | 62 | 71 |
| 2 | −16.2 | 6.5 | 0.7 | 83 | 99 |
| 3 | −14.5 | 5.7 | 0.7 | 74 | 97 |
| 4 | −7.5 | 6.2 | 0.8 | 66 | 85 |

**TABLE 2. Gradients in Repolarization Time (in Milliseconds)**

<table>
<thead>
<tr>
<th>Heart No.</th>
<th>Whole Heart</th>
<th>LV</th>
<th>Septum</th>
<th>RV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>16</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>23</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>23</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>24</td>
<td>6</td>
<td>20</td>
</tr>
</tbody>
</table>

LV and RV indicate left and right ventricle, respectively; ND, not determined.
Cardiac Memory and Regional Differences in Repolarization

Figure 5 shows epicardial activation times, ARIs, and repolarization times in 1 experiment in the control situation and after 2 hours of ventricular pacing. The epicardial surface of the ventricles is represented by sectors, as detailed in the legend. Repolarization times at right lateral and right posterior sites were least altered in cardiac memory, whereas other sites had shorter repolarization times after 2 hours of ventricular pacing. Because there were no differences in activation times, the changes in repolarization times were caused by changes in ARI.

In the absence of transmural gradients, we did observe 2 repolarization gradients in the left ventricle: 1 from base to apex, with shortest repolarization times at the base, and 1 from anterior to posterior, with shortest repolarization times at the anterior left ventricle. For the sake of simplicity, we show in Figure 6 repolarization times for all hearts at the base and apex and at anterior and posterior left ventricles. The shortest repolarization times are found at the base of the anterior left ventricle and the longest, at the apex of the posterior left ventricle, with 1 exception: In heart 2, the repolarization time at the apical posterior left ventricle is shorter than at the base. Nevertheless, repolarization in the anterior left ventricle occurred earlier at the base than at the apex, as in the other hearts. In hearts 1 through 3, repolarization always occurred earlier in the anterior left ventricle compared with the posterior left ventricle, both at the base and apex. In heart 4, however, posterior repolarization occurred somewhat earlier. Both before and after induction of memory, the observed gradient in repolarization from base to apex reached statistical significance (P=0.042 and 0.006, respectively). The gradient from anterior to posterior was borderline-significant (P=0.13 and 0.051, respectively).

Repolarization times in the right ventricle were considerably shorter than in the left ventricle or in the septum. In the right ventricle, repolarization occurred earlier at the base than at the apex. The gradient of repolarization in the septum was small when compared with the gradients in the right ventricle or in the left ventricular free wall. Septal repolarization consistently occurred later at the base than at the apex. Numerical data for the individual hearts are provided in Table 2.

Figure 7 shows monophasic APs from the epicardium in 1 experiment. APs were adjusted for amplitude, and their upstrokes were superimposed before and after induction of memory. AP shortening is most marked at the terminal part of repolarization. AP shortening after induction of memory is variable. This may be partly due to the fact that recordings of monophasic APs were made sequentially over a period that could be as long as 10 minutes. After 2 hours of ventricular pacing, the effects of memory gradually disappear, so that in different recordings, different degrees of memory may have been present. Still, AP shortening in the late repolarization phase is present in most recordings. For all recordings in the 4 hearts (n=48), the shortening was –1.3 ms at the 30% repolarization level, –2.6 ms at the 50% level, and –10.4 ms at the 90% level. This trend was statistically significant (P=0.016, repeated-measures analysis, test for trend). Also in the monophasic AP recordings there was an apicobasal
gradient in repolarization: When considering all left ventricular recordings, repolarization times at the 90% repolarization level in control conditions at the basal sites were 222±2.0 ms and after induction of memory, 213±4.9 ms. For apical sites, these values were 230±3.2 and 217±8.1 ms, respectively.

Epicardial isopotential maps were constructed at the moment of the nadir of the T wave in lead aVR. In 2 experiments, a sufficient number of electrograms had identical QRS amplitudes before and after memory (n=45 and 25, respectively). Electrograms after memory were often smaller or larger than in control conditions, and this could have been due to a change in contact of the epicardial electrodes during the course of the experiment. Isopotential maps of the 2 experiments revealed no significant change after the induction of memory (data not shown).

Discussion

Our findings can be summarized as follows: (1) We found no significant transmural gradient in repolarization times in the left ventricular wall or in the interventricular septum, either in control conditions or after 2 hours of ventricular pacing. (2) There was a left ventricular apicobasal gradient with shortest repolarization times in anterior basal regions and longest repolarization times in posterior apical regions. (3) Repolarization time, ARIs, and monophasic AP durations shortened after induction of short-term memory. This shortening was particularly evident at the 90% repolarization level and was ~10 ms.

In isolated cells and in vitro, the existence of M cells, having longer AP durations than epicardial or endocardial cells, is well documented. However, most studies of intact hearts have failed to provide evidence for a midmyocardial layer with longer repolarization times (see Anyukhovsky et al18 for review). This was the case for studies in which refractory periods were measured at various depths in the left ventricular wall19–23 and for studies in which ARIs or repolarization times were measured at intramural sites in intact canine or human hearts.15,24–26 Only the study by El-Sherif et al27 showed a significant but small prolongation of the ARI in midmural regions of the left ventricle in young dogs.

There have been reports that the type of anesthesia might determine whether or not M cells will be detected in in vivo studies. As summarized by Antzelevitch,28 studies with pento-barbital or α-chloralose found smaller transmural dispersion in repolarization than did studies in which isoflurane or halothane was used. We induced anesthesia with the ultrashort-acting barbiturate thiopental and maintained anesthesia with isoflurane. As argued by Taggart et al,25 “...isoflurane does cause a degree of cellular uncoupling and therefore produces optimal conditions for the detection of transmural repolarization gradients.” Despite this, we and Taggart et al25 detected no significant transmural gradients in repolarization. Because of the time needed to attach the epicardial and transmural electrodes and an equilibration period, the possible effects of thiopental have disappeared long before recordings were made.
Our study differs from previous ones on cardiac memory in that AP duration shortened rather than lengthened (for a review, see Patberg and Rosen29). The most likely explanation for this is as follows. Those studies showing a prolongation of AP duration made recordings from tissue slabs (endocardial, midmyocardial, epicardial) isolated 1 to 2 cm from the pacing electrode site in the hearts of dogs paced for at least 2 to 3 weeks to induce long-term memory. These changes in repolarization were associated with, and likely due to, a decreased amplitude of the transient outward current, Ito,7,8 as well as altered kinetics of Ito and ICa-L in left ventricular epicardial myocytes.8,9 In these epicardial tissue slabs, the “notch” of the AP, caused by Ito, decreased in the setting of long-term memory, and the transmural gradient measured in isolated tissues was altered as follows. Epicardial and endocardial AP durations both increased, with the epicardium more prolonged than endocardium, so that the gradient was reduced.7 In studies of short-term memory in intact animals, pharmacological blockade of Ito prevented the occurrence of memory.30 In that study, corrected QT intervals did not change with induction of short-term memory.

The only study of canine APs during short-term memory was performed not in intact animals but in an isolated tissue model, in which slabs of epicardium and endocardium were alternately paced along and perpendicular to the long myocardial fiber axis.31 In that study, a mock T wave was recorded with differential amplifiers, and the modeled cardiac memory was associated with increases in epicardial and endocardial AP durations. However, it must be emphasized that those studies were performed on isolated tissue slabs that remained for long intervals in the tissue bath, rather than in tissues that had been acutely isolated from hearts in situ with short-term memory. Although these results are not consistent with our present findings, the reasons for the differences likely reside in the very different model systems used. Certainly, the current model is the more relevant for the intact heart. Our finding that AP shortening is most pronounced at the 90% repolarization level is consistent with possible roles for I_{kr}, I_{ks}, or I_{K1}.

What Causes the Deepening of the T Wave in Short-Term Memory?

Although isopotential maps could be made only from a limited number of epicardial sites, we found no evidence for different potential gradients at the moment of the nadir of the T wave before and after induction of memory. Also, we found no change in the direction of the gradient in repolarization times. Figure 8 shows in a diagrammatic form how the steeper phase 3 of the AP after induction of memory could lead to a deepening of the T wave. Two APs are superimposed, with a relatively slow phase 3 (left) and a rapid phase 3 (right). At the moments indicated by the vertical lines, intracellular potentials are shown in 2 “cells” that are coupled to each other. The local current circuits are indicated, as well as the extracellular potential. Clearly, a steeper final repolarization phase could lead to a deepening of the extracellular T wave, the phenomenon demonstrated experimentally in Figure 1.

Limitations of the Study

Our observations are based on >100 measurements at as many as 60 sites in each of 4 hearts. We found great consistence in each of these hearts. The statistical analysis, based on repeated-measures analysis, used the 4 hearts as the experimental units and only marginally accommodated the consistence of the findings in each heart. This explains why some of the trends reached only borderline significance.

Acknowledgments

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Figure 8. Diagram to indicate how a steeper phase 3 of the AP after memory might lead to a deepening of the T wave: Superimposed APs in control conditions (left) and after memory (right) are shown together with 2 “cells” that are coupled. Intracellular potentials are shown at the moments of the 3 vertical lines, and arrows indicate local current circuits, which in the right panel lead to deeper negative extracellular potentials.

References


**CLINICAL PERSPECTIVE**

More than 100 years after Einthoven recorded the human ECG with his string galvanometer, there is still debate about the genesis of the T wave. The inverted polarity of the T wave compared with the QRS complex may be due to transmural and/or apicobasal gradients in repolarization times, with longer times to repolarization at the endocardium compared with the epicardium and at the apex compared with the base. Most authors consider the gradient across the ventricular wall as most important. We recorded unipolar electrograms from as many as 98 epicardial and 144 intramural sites from canine hearts. Activation times and activation-recovery intervals (ARIs) were measured before and after induction of short-term memory by 2 hours of ventricular pacing. After resuming atrial pacing, the effect of memory was evident as a deepening of the T wave, especially in leads II and aVF, and by a shortening of the QT interval. Also, epicardial monophasic action potentials were recorded. Activation times increased from the endocardium to the epicardium while ARIs decreased. Their sum (activation time + ARI = repolarization time) remained constant throughout the ventricular wall. In contrast, there was an apicobasal gradient, with shortest repolarization times in the anterobasal region and longest times in the posterobasal region. Induction of memory did not alter activation times but did shorten ARIs by ∼10 ms throughout the ventricular wall owing to shortening of the monophasic action potential at the 90% repolarization level. The more rapid phase 3 of the action potential may explain the deeper T wave induced by pacing. Thus, both in control conditions and after induction of cardiac memory, the T wave is determined by apicobasal gradients in repolarization time and not by transmural gradients.
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