Transient Outward Current Prominent in Canine Ventricular Epicardium but Not Endocardium

Silvio H. Litovsky and Charles Antzelevitch

Previous studies have denied the presence of a transient outward current (I_o) in ventricular myocardium of dog, sheep, and calf. Using conventional microelectrode techniques, we provide evidence for a significant contribution of I_o to epicardial, but not endocardial, activity of canine ventricular myocardium. The epicardial action potential when compared with that of endocardium shows a smaller phase 0 amplitude, a much more prominent phase 1, and a phase 2 amplitude that is greater than that of phase 0. Epicardial action potentials, unlike those of endocardium, display a “spike and dome” morphology that becomes progressively more accentuated at slower stimulation rates. Using the restitution of phase 1 amplitude as a marker for the process responsible for the spike and dome phenomenon, we were able to delineate two exponential components: 1) a slow component that recovers with a time constant of 350–570 msec and 2) a fast component with a time constant of 41–85 msec. The slow component was largely abolished by 1–5 mM 4-aminopyridine, an I_o blocker. The fast component was diminished by 4-aminopyridine, but it was also inhibited by ryanodine and by Sr^2+ replacement of Ca^2+, which are interventions known to inhibit the Ca^2+-activated component of I_o. Following 4-aminopyridine and Sr^2+ or ryanodine treatment, the epicardial responses more closely resembled those of endocardium. In summary, the data demonstrate a marked heterogeneity of active membrane properties in canine ventricular muscle. These observations may aid in understanding the basis for rate-dependent changes in the T wave of the ECG, supernormal conduction in ventricular muscle, the greater sensitivity of epicardium to ischemia, and the rate dependence of some cardiac arrhythmias. (Circulation Research 1988;62:116–126)

The transient outward current (I_o) has been described in several cardiac and noncardiac tissues and has been assigned a number of different names including “positive dynamic current,” “chloride current,” “initial outward current,” “I_o,” and “early outward current.” In the heart, its presence has been reported in sheep, calf, and dog Purkinje fibers, rabbit and rat ventricular myocardium, and human atrial tissues. Recent studies have also described this current in isolated rat ventricular myocytes, rabbit atrioventricular nodal cells, crista terminalis cells, and canine Purkinje cells. I_o 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 Cohen et al. for review).

The I_o is thought to be largely absent in canine, sheep, and calf myocardium. Beeler and Reuter, in their reconstruction of the action potential of myocardial fibers, noted that “this current component does not appear to be present to any significant degree in ventricular myocardial preparations.” It is noteworthy that almost all voltage-clamp studies devoted to myocardial currents, and I_o in particular, have employed endocardial tissues (either papillary muscles or trabeculae). The absence of I_o in these tissues, however, has been generalized to the entire ventricular mass.

In comparing transmembrane activity recorded from the endocardial and epicardial surfaces of the ventricles of various species, several obvious differences may be discerned. Chief among these is the manifestation of a “spike and dome” morphology in transmembrane action potentials recorded from ventricular epicardium but absent in endocardium. This difference has also been observed during simultaneous recordings of monophasic action potentials from the epicardial and endocardial surfaces of the canine heart in vivo. The spike and dome configuration observed in epicardial cells stems from a steep and prominent phase 1. Because I_o has been shown to contribute to the manifestation of phase 1 in canine and sheep Purkinje fibers, we considered the hypothesis that I_o may be prominent in epicardial, but not endocardial, tissues and that this difference may in large part account for the different morphologies of the action potential and other characteristic differences in the behavior of the two tissue types.

The present study was designed to test this hypothesis and to provide further characterization of the differences that exist between the electrical behavior of endocardial and epicardial tissues of the canine ventricle.

**Materials and Methods**

Papillary muscles and right ventricular epicardial strips (approximately 2.0 x 1.5 x 0.2 cm) were isolated...
from hearts removed from anesthetized (sodium pentobarbital, 30 mg/kg) mongrel dogs of either sex. The epicardial preparations were obtained from razor blade shavings (Davol Simon Dermatome Power Handle No. 3293 with cutting head No. 3295, Cranston, R.I.) made parallel to the fiber orientation in the right ventricular free wall. In 5 experiments, endocardial strips were similarly isolated from the right ventricular free wall. Because we found no significant differences between the characteristics of the shaved endocardial preparations and those of the papillary muscles, we grouped these together in the "Results." It might also be noted that no difference could be discerned between the activity of intact papillary muscles and of strips shaved from the surface of these muscles (2 preparations). The use of the terms "endocardial" and "epicardial" in this report refer to the myocardial cells on the respective surfaces of the ventricular wall, representing the outermost subendocardial and subepicardial layers.

Epicardial and endocardial preparations from the same heart were placed in a tissue bath and allowed to equilibrate for 1 hour while superfused with an oxygenated (95%O₂, 5%CO₂) Tyrode’s solution (37 ± 0.5°C; pH 7.35). Unless otherwise indicated, the composition of Tyrode’s solution was (in mM) NaCl 129, KCl 4, NaH₂PO₄ 0.9, NaHCO₃ 20, CaCl₂ 1.8, MgSO₄ 0.5, and D-glucose 5.5.

The tissues were stimulated at basic cycle lengths (BCL) ranging from 200 to 4,000 msec using rectangular stimuli (1–3 msec duration, 2.5 times diastolic threshold intensity) delivered through silver bipolar electrodes 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 (WP Instruments, New Haven, Conn.). Amplified signals were displayed on an oscilloscope (Tektronix, Beaverton, Ore.) and photographed on a 35-mm kymographic camera (Grass, Quincy, Mass.) or recorded on FM tape (A.R. Vetter Co., Rebersburg, Penn.). The maximal rate of rise of the action potential upstroke was measured with a differentiator adjusted for linearity within the range of 50–500 V/sec.

Restitution of action potential characteristics was determined using single test pulses (S₂) delivered after every tenth basic beat (S₁). The S₁-S₂ coupling interval was increased progressively from the end of the refractory period until the next basic beat.

4-Aminopyridine (4-AP) (Sigma Chemical Co., St. Louis, Mo.) 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. Because 4-AP has been reported to cause release of neurotransmitters from adrenergic and cholinergic nerve endings, the effect of a combination of propranolol (0.3 μg/ml), phentolamine (1.0 μg/ml), and atropine (1.0 μg/ml) was assessed in the initial experiments. Use of these agents was discontinued once it was determined that they did not alter the actions of 4-AP. Ryanodine (Merck Sharp & Dohme, Rahway, N.J.) was prepared as a stock solution of 1.0 mM.

Statistical analysis was performed using the Student’s t test for paired or unpaired data as indicated.

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**Figure 1.** Transmembrane action potentials recorded from an epicardial strip (top panel) and a papillary muscle (bottom panel). Basic cycle length (BCL) = 500 msec. Upstrokes retouched in this and subsequent figures.
Table 1. Action Potential Parameters of Epicardial and Endocardial Preparations

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<tr>
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<th>Epicardium</th>
<th>Endocardium</th>
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<tbody>
<tr>
<td>Resting potential (mV)</td>
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<td>82.9±1.8 (17)</td>
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<tr>
<td>Amplitude</td>
<td></td>
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<tr>
<td>Phase 0 (mV)</td>
<td>91.3±5.0 (24)</td>
<td>106.6±2.9 (17)†</td>
</tr>
<tr>
<td>Phase 1 (mV)</td>
<td>76.9±6.1 (24)</td>
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<td>Phase 2 (mV)</td>
<td>91.2±5.0 (24)</td>
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<td>Magnitude</td>
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<tr>
<td>Phase 1 (mV)</td>
<td>14.3±5.6 (24)</td>
<td>7.4±2.9 (17)†</td>
</tr>
<tr>
<td>APD₉₀ (msec)</td>
<td>117.0±11.4 (24)</td>
<td>130.0±13.7 (17)*</td>
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<tr>
<td>APD₉₅ (msec)</td>
<td>143.8±12.6 (24)</td>
<td>164.0±13.6 (17)†</td>
</tr>
<tr>
<td>dV/dtₘ₅ₓ (V/sec)</td>
<td>199.1±25.6 (7)</td>
<td>178.1±41.9 (7)</td>
</tr>
<tr>
<td>Excess overshoot (mV)</td>
<td>6.5±2.9 (24)</td>
<td>0.6±0.3 (17)†</td>
</tr>
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</table>

APD₉₀ and APD₉₅ are action potential durations measured at 50 and 90%, respectively, of full repolarization. dV/dtₘ₅ₓ is maximum rate of rise of action potential upstroke.

All values are given as mean ± SD. Numbers in parentheses represent number of preparations. Measured at a basic cycle length of 500 msec.

* p<0.05; † p<0.01. Significance determined by unpaired Student’s t test for differences between endocardium and epicardium.

Results

Figure 1 illustrates representative transmembrane activity recorded from an epicardial strip (top) and a papillary muscle, both stimulated at a BCL of 500 msec. The epicardial action potential when compared with that of the endocardium shows a smaller phase 0 overshoot (8 mV versus 24 mV), a much more prominent phase 1 (phase 1 magnitude: 19 mV versus 6 mV), a phase 2 amplitude greater than that of phase 0, and a briefer action potential duration (measured at 90% repolarization; APD₉₀: 135 msec versus 150 msec). The resting potential and maximal rate of rise of the action potential upstroke were not significantly different in the two tissue types (Table 1).

This morphology of the epicardial action potential was found in 38 of 40 experiments; no major qualitative differences were noted between recordings obtained from the base and apex of the right ventricle (3 experiments). In 2 preparations, action potentials were recorded without a prominent spike and dome. We have no definitive explanation for this finding other than to suggest that these observations may be related to the age of the animals. Previous studies have shown progressive age-related development of the spike and dome in human atrial tissue and in canine Purkinje fibers. The two preparations in question were obtained from relatively young dogs.

Because a spike and dome configuration could be related to discontinuities in conduction (regions of depression or anisotropy), we mapped the preparations in initial experiments to compare intracellular activity at different sites and also evaluated the effect of different stimulation protocols and of alteration of the stimulation site. The following observations argue against the possibility that epicardial spike and dome morphology is due to a conduction artifact: 1) prepotentials were never observed; 2) the spike and dome morphology was lost when the tissue depolarized (2 preparations); 3) the morphology was less evident with premature beats, a situation in which one would expect the spike and dome to be more pronounced if it were secondary to a conduction disturbance; 4) similar morphologies were generally re-

Figure 2. Restitution of action potential parameters in epicardium (top panels) and endocardium (papillary muscle) (bottom panels). BCL = 2,000 msec. Time dependence of the spike and dome morphology in epicardium becomes more evident at slower basic stimulation rates. Note also the biphasic restitution of total amplitude in epicardium and absence of this phenomenon in endocardium.
corded throughout the preparation, and the stimulation site did not significantly alter the activity; and 5) simultaneous stimulation of two different sites, no matter their location, did not significantly modify the action potential morphology.

Restitution of action potential parameters in epicardium and endocardium is illustrated in Figure 2. Each panel shows a composite of 6–9 sweeps of the oscilloscope. The first response in each panel is the last of a train of 10 basic beats; subsequent beats represent the responses to premature stimuli applied progressively later in the cycle. In epicardium, the amplitudes of phases 0, 1, and 2 of premature beats elicited early in diastole were greater than those of the basic beats. These changes were attended by a disappearance of the spike and dome morphology so that the action potential morphology of early premature beats in epicardium resembles those of endocardium. Similar changes were observed in the basic responses when the stimulation rate was accelerated. In the example shown, the spike and dome configuration of epicardium was greatly attenuated when the stimulation rate was changed from a BCL of 2,000 msec to 400 msec. The morphology of the endocardial action potential was clearly less rate-dependent; aside from the changes in action potential duration, the restitution scan revealed only slight changes in other parameters.

Another prominent distinction is that the restitution of phase 0 amplitude is biphasic in epicardium but monotonic in endocardium. In both tissue types, the earliest response that could be elicited showed a phase 0 amplitude that was smaller than that of the basic beat. In endocardium, the phase 0 amplitude of later beats quickly approached that of the basic beat. In epicardium, however, the phase 0 amplitude (overshoot) of later premature beats was distinctly larger than that of the basic beat. This excess overshoot in early diastole was a constant finding in epicardium (Table 1) but was rarely present in endocardium. When present, it never exceeded 2 mV.

**Effect of 4-Aminopyridine**

Because the spike and dome morphology and the rate-dependent changes observed in the epicardial action potential were suggestive of the presence of $I_{\text{to}}$, we examined the effects of 4-AP, a transient outward current blocking agent. A representative experiment is pictured in Figure 3. Under control conditions (Panel A), the restitution scan (BCL = 2,000 msec) produced results similar to those described in Figure 2. Thirty minutes after the addition of 1 mM 4-AP (Panel B), endocardial activity showed a prolonged action potential duration with little change in the other action potential parameters. In epicardium, 4-AP produced a prominent augmentation of phase 0 and phase 1 amplitudes and greatly attenuated the spike and dome morphology. Panel C, recorded 20 minutes after increasing the concentration of 4-AP to 5 mM, shows a further augmentation of the amplitudes of phases 0 and 1 in epicardium and a loss of time-dependent

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**Figura 3.** Effect of 4-aminopyridine (4-AP), a transient outward current blocking agent, on restitution of action potential parameters in endocardium (right panels) and epicardium (left panels). A, Control. B, Recorded 30 minutes after addition of 1 mM 4-AP; spike and dome morphology and time-dependent characteristics of epicardium are greatly attenuated. C, Recorded 20 minutes after increasing the 4-AP concentration to 5 mM. Phase 0 and phase 1 amplitudes of epicardial responses are further increased, and their time dependence is largely abolished. 4-AP produces no change of these parameters in the activity recorded from endocardium. BCL = 2,000 msec.
Table 2. Effect of 1 mM 4-Aminopyridine on Action Potential Parameters of Epicardium and Endocardium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4-AP</th>
<th>Difference</th>
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<tbody>
<tr>
<td><strong>Epicardium (n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Resting potential (mV)</td>
<td>81.9 ± 0.9</td>
<td>81.6 ± 0.7</td>
<td>-0.3 ± 0.5</td>
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<tr>
<td>Amplitude</td>
<td></td>
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<tr>
<td>Phase 0 (mV)</td>
<td>88.9 ± 4.7</td>
<td>93.2 ± 3.7</td>
<td>4.3 ± 2.5†</td>
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<td>Phase 1 (mV)</td>
<td>61.6 ± 5.4</td>
<td>86.9 ± 1.8</td>
<td>25.3 ± 6.0†</td>
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<tr>
<td>Phase 2 (mV)</td>
<td>95.3 ± 5.4</td>
<td>91.4 ± 3.5</td>
<td>-3.9 ± 5.6</td>
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<td>Magnitude</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1 (mV)</td>
<td>27.3 ± 5.4</td>
<td>6.3 ± 3.9</td>
<td>-21.0 ± 4.9†</td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>152.2 ± 22.2</td>
<td>136.0 ± 19.1</td>
<td>-16.2 ± 13.8*</td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>178.9 ± 26.8</td>
<td>165.8 ± 21.8</td>
<td>-13.1 ± 12.2*</td>
</tr>
<tr>
<td>Excess overshoot (mV)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(phase 0)</td>
<td>7.9 ± 3.2</td>
<td>4.0 ± 2.5</td>
<td>-3.9 ± 1.7†</td>
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<td><strong>Endocardium (n = 8)</strong></td>
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<td>Resting potential (mV)</td>
<td>82.0 ± 1.6</td>
<td>81.5 ± 1.1</td>
<td>-0.5 ± 1.4</td>
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<td>Amplitude</td>
<td></td>
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<tr>
<td>Phase 0 (mV)</td>
<td>103.0 ± 2.9</td>
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<td>Phase 1 (mV)</td>
<td>94.7 ± 1.5</td>
<td>98.8 ± 2.2</td>
<td>4.1 ± 2.1*</td>
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<td>Magnitude</td>
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<tr>
<td>Phase 1 (mV)</td>
<td>8.2 ± 1.7</td>
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<td>APD90 (msec)</td>
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<td>APD90 (msec)</td>
<td>177.8 ± 16.4</td>
<td>194.2 ± 24.2</td>
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<td>Excess overshoot (mV)</td>
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<tr>
<td>(phase 0)</td>
<td>0.7 ± 1.0</td>
<td>0.1 ± 0.2</td>
<td>-0.7 ± 1.0</td>
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</table>

APD90 and APD90 are action potential durations measured at 90 and 90%, respectively, of full repolarization. 4-AP, 4-aminopyridine.

All values are given as mean ± SD measured at a basic cycle length of 2,000 msec. n, Number of preparations.

Two Components for the Recovery of Phase 1 Amplitude

Recovery characteristics of phase 1 amplitude were evaluated in 11 preparations. Figure 4 illustrates the results of a representative experiment. Each panel shows a semi-logarithmic plot of the difference between the phase 1 amplitude of the basic beat (BCL = 2,000 msec) and that of premature beats elicited at progressively longer S1-S2 intervals (Method I). Under control conditions (Panel A), two exponential components were apparent: 1) a slow component that decreased with a time constant of 500 msec and 2) a faster component that decayed with a time constant of 70 msec. The addition of 1 mM 4-AP (Panel B, 30 minutes) produced little change in the time constants of the two processes but decreased the maximum values (extrapolated to the start of diastole) of the slow and fast components to 18 and 33% of their control values, respectively. An increase of [4-AP] to 5 mM (Panel C, 20 minutes) completely abolished the slow component and further diminished the fast component to 12.9% of its control value; its time constant remained unchanged.

The time dependence of decay of Δphase 1 amplitude appears consistent with the time dependence of reactivation of INa observed in other studies. A large difference between the phase 1 amplitude of an early premature beat and that of the basic beat is expected because little recovery of INa would have occurred. As recovery of INa progresses, the phase 1 amplitude difference should diminish as the spike and dome morphology progressively recovers with later beats. The delineation of two exponential processes with brief and long time constants and the greater sensitivity of the slower component to 4-AP are observations consistent with previous reports describing the effect of this agent on INa in other tissues.

In 4 experiments in which 4-AP (1 mM) largely abolished the spike and dome morphology of the epicardial action potential, we obtained a direct measure of the 4-AP–sensitive components by evaluating the difference between the phase 1 amplitude recorded before and after 4-AP (Method II). The results of one such experiment are illustrated in Figure 5.

In this format, the 4-AP–sensitive component(s) is represented by the voltage difference of the phase 1 amplitude of responses recorded before and 30 minutes after the introduction of 1 mM 4-AP (Figure 5, top inset). The difference voltage is plotted on a logarithmic scale as a fraction of the maximum 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 panel illustrates the method used to derive the time constants. The results were well fitted by two exponential processes, and the time constants for the recovery of the two components were similar to those obtained with Method I.

Table 3 presents the results of 11 experiments in which the recovery time constants were evaluated using Methods I and II. Paired statistical analysis of the data from the five experiments in which both methods were
Figure 4. Recovery of phase 1 amplitude in epicardium. Each panel shows a semilogarithmic plot of the difference between phase 1 amplitude of the basic beat (BCL = 2,000 msec) and that of premature beats introduced at progressively longer S1-S2 intervals (once after every 10th basic response) (see inset). The Δphase 1 amplitude (○) is plotted on a logarithmic scale so that an exponential decline provides a straight line. Curves fitted by eye.

A. Under control conditions, two exponential components are apparent. The slow component decreases with a time constant (τ) of 500 msec (Δphase 1 amplitude = 13 mV). Subtraction of this component from the measured value yields a second exponential process (●) that decays with a τ of 70 msec (amplitude = 2.4 mV).

B. Obtained 30 minutes after the addition of 1 mM 4-AP. The intensity of the slow and fast components diminished to 33% and 18% of control, respectively. (Amplitudes of slow and fast components were 2.4 and 5.9 mV.)

C. Measured 20 minutes after increasing the concentration of 4-AP to 5 mM. The slow component was abolished, and the faster component decreased to 12.9% of its control value (amplitude = 2.3 mV).

Calcium-Activated Component

The fast (brief) component of I\textsubscript{o} has been shown to be Ca\textsuperscript{2+}-activated.\textsuperscript{48-9} Replacement of Ca\textsuperscript{2+} by Sr\textsuperscript{2+} or exposure to caffeine, ryanodine, or calcium blockers is known to reduce this component of the transient outward current in other tissues. Accordingly, we evaluated the effects of ryanodine (1 μM) and Sr\textsuperscript{2+} replacement of Ca\textsuperscript{2+} on epicardial activity in 3 experiments (Figure 6). Both interventions greatly diminished the fast component but produced little effect on the slow exponential process. As illustrated in Figure 6, Sr\textsuperscript{2+} (1.8 mM) replacement of Ca\textsuperscript{2+} decreased the magnitude of the fast component to 43% of its control value (left panels), but ryanodine decreased this component to 57% of control. Although the time constants of the fast process remained unchanged, those of the slow process were prolonged. In all cases, the spike and dome morphology was considerably less prominent (4 experiments).

Restitution of Phase 0 Amplitude in Epicardium

Under control conditions, the recovery of phase 0 amplitude also shows a rapid phase followed by a slower one. When evaluated in a semilogarithmic manner, the recovery of phase 0 amplitude shows a biexponential fit with time constants similar to those obtained for the slow component. The Table 3. Time Constants for Recovery of Phase 1 Amplitude

<table>
<thead>
<tr>
<th>Experiment</th>
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<th>Long</th>
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</table>

| 111 | 70 | 500 |

66.0 ± 13.1  452.3 ± 71.5  55.4 ± 12.8  473.0 ± 103.8

Method I is described in Figure 4. Method II is described in Figure 5.

All values given in milliseconds.
processes with time constants comparable to those obtained from epicardium suggested otherwise. A spike and dome transmembrane action potentials recorded from canine epicardium plotted as a function of the diastolic interval, i.e., the time for reactivation), the transient outward current was eliminated by exposure to 5 mM 4-AP. Similar results were obtained in four other experiments.

**Discussion**

Although the presence of a transient outward current in canine myocardium has generally been denied, the manifestation of a spike and dome morphology of transmembrane action potentials recorded from canine epicardium suggested otherwise. A spike and dome configuration has generally been ascribed to 1) a step delay of conduction or 2) a contribution of a rapidly activating transient outward current to the active membrane properties of the cell.

In preliminary studies, we ruled out the possibility that this phenomenon was secondary to the conduction characteristics of epicardial tissues. Alternatively, we present evidence in support of a significant \( I_w \) contribution to the electrical activity of the epicardium but not endocardium of the canine ventricle.

**Rate and Time Dependence**

As previously reported for other tissues, the spike and dome morphology of the canine epicardial action potential is highly rate- and time-dependent (Figure 2). Rapid stimulation (BCL \( \leq 300 \) msec) greatly attenuates the magnitude of the characteristic notch but does not eliminate it. The spike and dome morphology is totally absent in closely coupled premature beats, and its recovery is largely, but not totally, complete within 2,000 msec. The recovery process is characterized by a relatively slow restitution of the amplitudes of phases 0, 1, and 2 of the action potential. In endocardium, these phases show little, if any, rate or time dependence. These observations may be explained by the presence of a rapidly activating, but slowly reactivating, outward current in epicardium that is largely absent in endocardium. Reports of similar time-dependent characteristics in other tissues have implicated the transient outward current.

**Evidence for Two Components**

Because the phase 1 amplitude of the epicardial action potential showed the greatest time-dependent changes, this parameter was used to quantitate the kinetics of the recovery process (Figures 4 and 5). The restitution of phase 1 amplitude was well fitted by two exponential processes with time constants comparable to those obtained using the method shown in Figure 4. All curves fitted by eye.

As illustrated in Figure 8, 1 mM 4-AP eliminated the slow component but not the fast. At a concentration of 5 mM, 4-AP abolished both components. Note that at an \( S_1-S_2 \) interval of 200 msec, at a time when \( I_w \) might be expected to be near minimum (due to insufficient time for reactivation), the transient outward current blocking agent (4-AP) exerts little if any effect. Also noteworthy is the biphasic restitution of phase 0 amplitude (control). The decline at the shortest \( S_1-S_2 \) intervals is due to encroachment of the premature beat on phase 3 of the basic response. The phase of excess overshoot, clearly apparent in control, is totally eliminated by exposure to 5 mM 4-AP. Similar results were obtained in four other experiments.

**Discussion**

Although the presence of a transient outward current in canine myocardium has generally been denied, the manifestation of a spike and dome morphology of transmembrane action potentials recorded from canine epicardium suggested otherwise. A spike and dome configuration has generally been ascribed to 1) a step delay of conduction or 2) a contribution of a rapidly activating transient outward current to the active membrane properties of the cell.

In preliminary studies, we ruled out the possibility that this phenomenon was secondary to the conduction characteristics of epicardial tissues. Alternatively, we present evidence in support of a significant \( I_w \) contribution to the electrical activity of the epicardium but not endocardium of the canine ventricle.

**Rate and Time Dependence**

As previously reported for other tissues, the spike and dome morphology of the canine epicardial action potential is highly rate- and time-dependent (Figure 2). Rapid stimulation (BCL \( \leq 300 \) msec) greatly attenuates the magnitude of the characteristic notch but does not eliminate it. The spike and dome morphology is totally absent in closely coupled premature beats, and its recovery is largely, but not totally, complete within 2,000 msec. The recovery process is characterized by a relatively slow restitution of the amplitudes of phases 0, 1, and 2 of the action potential. In endocardium, these phases show little, if any, rate or time dependence. These observations may be explained by the presence of a rapidly activating, but slowly reactivating, outward current in epicardium that is largely absent in endocardium. Reports of similar time-dependent characteristics in other tissues have implicated the transient outward current.

**Evidence for Two Components**

Because the phase 1 amplitude of the epicardial action potential showed the greatest time-dependent changes, this parameter was used to quantitate the kinetics of the recovery process (Figures 4 and 5). The restitution of phase 1 amplitude was well fitted by two exponential processes with brief (41–85 msec) and long (350–570 msec) time constants (Table 2). In one method, we evaluated the recovery process by comparing the phase 1 amplitude of the premature beats to the phase 1 amplitude of the basic beat (BCL = 2,000 msec), the latter representing full (or nearly full) recovery. In another, we measured recovery as the "difference voltage" in the phase 1 amplitude of the premature responses produced by exposure to 4-AP (see below). Both methods yielded qualitatively similar results. Moreover, similar results were obtained using phase 0 amplitude as a marker for the recovery process.

The delineation of two components with brief and long time constants is consistent with the results of voltage-clamp studies of \( I_w \) in other cardiac tissues. Although a long reactivation time is a known feature of \( I_w \), comparable data on the kinetics of this process are scarce.

Fozzard and Hiraoka found that recovery of this current (which they termed the "positive dynamic current") in sheep Purkinje fibers could be described by a single exponential with a time constant of about 500 msec at a potential of –84 mV. The authors noted, however, that careful analysis of their data revealed that the time course of reactivation could not be well fitted.
by a single exponential. Our analysis of their Figure 11 indeed revealed a good biexponential fit delineating a fast (time constant = 125 msec) and a slow (time constant = 1,200 msec) component ($V_r = -84$ mV). The time constants of both components prolonged greatly at progressively more positive voltages. At $-47$ mV, the contribution of the slow component was difficult to resolve because its time constant prolonged greatly relative to the duration of the test protocol and also because of a high degree of steady-state inactivation of the current.

The strong voltage dependence of $I_{\text{to}}$ may also account for the definition of only one component of $I_{\text{to}}$ in calf Purkinje fibers reported by Siegelbaum and Tsien.\cite{1} At their holding potential of $-50$ mV and at a test protocol extending to only 3 seconds, the slow component might not be expected to appear. In preliminary studies using techniques similar to those described in this paper, we have been able to discern two components for the recovery of phase 1 amplitude (normal resting potential) in calf Purkinje fibers (S. Litovsky and C. Antzelevitch, unpublished observation). A recent preliminary report by Kenyon and Sutko\cite{9} also concludes that there are two $I_{\text{to}}$ components in calf Purkinje fibers.

Giles and Van Ginneken\cite{16} have recently reported two components for reactivation of this current (which they term "$I_{\lambda}\)") in isolated rabbit crista terminalis cells with time constants of 100–150 msec and 1,000–3,000 msec ($-60$ to $-80$ mV). At potentials positive to $-60$ mV, the recovery was a single exponential representing the brief component.

The presence of two components of $I_{\text{to}}$ has now been described in a large variety of cardiac tissues and cells.\cite{3,14,15,16} An exception is the study of Josephson and coworkers\cite{14} using isolated rat ventricular cells (one component: reactivation time constant $= 25$ msec).

**Inhibition of Transient Outward Current**

The effect of 4-AP to inhibit both components of the recovery of phase 1 amplitude, with preferential inhibition of the slow component, is also in accord
with the effects of this agent on the two components of $I_w$, as demonstrated in voltage-clamp studies. Also consistent are the results obtained with ryanodine, an agent that inhibits Ca$^{2+}$ release from the sarcoplasmic reticulum, and with Sr$^{2+}$ replacement of Ca$^{2+}$, both showing selective inhibition of the fast component. These data suggest that in canine epicardium, as in several other cardiac tissues, the fast component of $I_w$ is Ca$^{2+}$ activated.

**Transient Outward Current Absent in Endocardium**

The following observations argue for a lack of an important contribution of $I_w$ to the electrical activity of canine endocardium: 1) absence of a spike and dome morphology; 2) lack of a prominent 4-AP effect on the early phases of the endocardial action potential; and 3) lack of significant time and rate dependence of the early phases of the action potential. The absence of a spike and dome configuration per se does not necessarily exclude the possibility of a $I_w$ contribution because this feature is lacking in tissues in which $I_w$ is relatively slow to inactivate. Time- and rate-dependent changes of the amplitude of the early phases of the action potential, however, occur in all tissues known to possess a $I_w$ and are secondary to the rapid activation and slow reactivation kinetics characteristic of this current. The effect of 4-AP to prolong the canine endocardial action potential may be largely due to an inhibition of a time-independent outward current. This effect is consistent with our preliminary observations of a parallel shift of the action potential duration–rate relation in endocardium following 4-AP (S. Litovsky and C. Antzelevitch, unpublished observation).

**Transient Outward Current in Canine Epicardium**

Taken together, the results support the hypothesis that a transient outward current contributes significantly to the electrical activity of canine epicardium but not of endocardium. A rather prominent heterogeneity of active membrane properties appears to exist among cells spanning the ventricular wall of the canine heart. The data also suggest that the restitution of phase 1 amplitude may be a reasonable marker for the quantitation of $I_w$ in this tissue.

Our results may have relevance to a recent preliminary report by Tseng and coworkers that describes two components of $I_w$ in canine ventricular myocytes (isolated from transmural segments of free wall). The authors indicate that only 64% of the cells showed a spike and dome configuration and that two rapidly activated components of $I_w$ could be discerned in these cells; one was inhibited by 4-AP and the other inhibited by Mn$^{2+}$ or caffeine but enhanced by elevated [Ca$^{2+}$].

**Role of Other Currents**

In view of the fact that in cardiac muscle $I_w$ is the only time-dependent current known to be blocked by 4-AP, the concordance of the results obtained using Methods I and II for characterization of the restitution of phase 1 amplitude suggests that a major contribution from a time-dependent current other than $I_w$ is unlikely. Of particular interest would be a contribution of the slow inward current, $I_{in}$, because this current is prominent during the spike and dome phase of the action potential. The lack of an effect of 4-AP on $I_{in}$, as demonstrated in other tissues, suggests a lack of a major contribution of $I_{in}$ to the recovery process, but a final determination must await voltage-clamp studies of canine epicardium.

The available data suggest that the elimination by 4-AP of the spike and dome and of time-dependent changes of phase 1 amplitude in epicardium is due largely to inhibition of $I_w$. This notwithstanding, it might...
be hypothesized that the absence of a spike and dome configuration in epicardium is due not to a difference in \( I_w \) contribution but rather to a difference in the level of \( I_n \). In this schema, a stronger \( I_n \) in endocardium may completely overwhelm any existing \( I_w \). Several arguments can be leveled against this hypothesis; chief among these are 1) voltage-clamp studies of various mammalian endocardial preparations have failed to uncover any significant contribution of \( I, \) 2) in the case of rabbit endocardium, the presence of \( I_n \) is associated with a time and rate dependence of the early phases of the action potential and with 4-AP sensitivity of these phases (lacking in canine endocardium); and 3) calcium channel blocking agents, including verapamil and cobalt, fail to induce a spike and dome in canine endocardium although they accentuate the spike and dome in canine epicardium (S. Litovsky and C. Antzelevitch, unpublished observation).

**Physiologic Significance**

One consequence of a \( I_n \) presence in epicardium is the manifestation of a biphasic restitution of the amplitude of phase 0 and phase 1 of the action potential, leading to an "excess overshoot" in early diastole (Figures 2, 3, and 8). Because the excess overshoot provides for a greater source current in early versus late diastole, a supernormal period of conduction may result under conditions of generally impaired conduction. Indeed, in preliminary experiments, we have succeeded in demonstrating a supernormal phase of conduction in canine epicardial strips mounted in a sucrose gap (H. Vetulli and C. Antzelevitch, unpublished observation).

The strong presence of \( I_n \) in epicardium but not in endocardium may also provide for a heterogeneous rate dependence of the action potential duration and effective refractory period. In another corollary study, we have found that the dependence of the action potential duration on stimulation rate is significantly more pronounced in epicardium than in endocardium of the canine ventricle. A crossover of the relation is often encountered so that at relatively short cycle lengths, the action potential duration and effective refractory period of endocardium is longer than that of epicardium, but at long cycle lengths, the converse is true. Exposure to 4-AP reduces the rate dependence of the action potential duration in epicardium, making it similar to that of endocardium. These relations should add to the understanding of rate-dependent changes in the T wave of the ECG and are likely to have some implications relative to our understanding of the rate dependence of cardiac arrhythmias.

Finally, the presence of an additional outward current in epicardium may account in part for the different sensitivities of epicardium and endocardium to depression under "ischemic" conditions. Recent studies by Gilmore and Zipes and Kimura and coworkers have demonstrated a lower tolerance of epicardium to ischemia in the canine and feline heart. Investigations are underway to test this hypothesis. Our preliminary findings indicate that \( I_n \) plays a major role in the selective depression of epicardium (A. Lukas and C. Antzelevitch, unpublished observation).

In summary, this study demonstrates a marked heterogeneity of active membrane properties in canine ventricular muscle. These data will hopefully prompt further investigations into the ionic basis of disparate electrical activity in different regions of the ventricular myocardium.

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**References**


KEY WORDS • transient outward current • endocardium • epicardium • endocardium • canine ventricle
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