Sodium Channel Block Produces Opposite Electrophysiological Effects in Canine Ventricular Epicardium and Endocardium

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Using microelectrode techniques we compared the effects of tetrodotoxin (TTX, 2–3 μM), DL-propranolol (1–3 μg/ml), and flecainide acetate (10–15 μM) on isolated canine ventricular epicardial (epicardium) and endocardial (endocardium) tissues. Propranolol, TTX, and flecainide decreased Vmax and phase 0 amplitude in a use-dependent manner in both tissues. The effects of propranolol were slow to develop and wash out. TTX and propranolol always abbreviated action potential duration in endocardium. Action potential duration was abbreviated by 23.8±5.6 msec after propranolol (1 μg/ml, basic cycle length [BCL]=1,000 msec) and 10.8±12.9 msec after TTX (2 μM, BCL=1,000 msec). In epicardium, the reduction of phase 0 and 1 amplitudes led to a slowing of the second action potential upstroke and an increase in the amplitude of phase 2. This accentuation of the notch resulted in a paradoxical prolongation of the epicardial action potential. Action potential duration was prolonged 34.4±11.3 msec after 4 hours of exposure to propranolol (1 μg/ml, BCL=1,000 msec), 11.1±6.3 msec after 15 minutes of exposure to TTX (2 μM, BCL=1,000 msec), and 19.9±8.2 msec after 25–45 minutes of exposure to flecainide (15 μM, BCL=500 msec). With stronger sodium block, phase 1 terminated at more negative potentials, the second upstroke often failed to appear, and an all-or-none repolarization ensued causing a marked abbreviation of the epicardial action potential. In some epicardial preparations, we observed marked abbreviation at some sites but prolongation at other sites after sodium blockade with flecainide. The dispersion of repolarization was often attended by reentrant activity. The differential response of epicardium and endocardium to sodium blockade was not observed when the preparations were pretreated with 4-aminopyridine or ryanodine, agents known to diminish the transient outward current and epicardial notch. Acceleration-induced prolongation of refractoriness was observed after sodium blockade in epicardium but not in endocardium. Postrepolarization refractoriness also occurred in epicardium but not in endocardium after TTX, propranolol, or flecainide exposure. The data indicate that propranolol, TTX, and flecainide, via their action to block sodium current, may exert opposite effects on action potential duration and refractoriness in cells spanning the ventricular wall. The presence of the transient outward current in epicardium but not in endocardium appears to contribute importantly to these differences. The differential responsiveness of ventricular myocardial tissues to sodium channel block demonstrated in this study will hopefully advance both our understanding and appreciation of the antiarrhythmic and arrhythmogenic consequences of sodium current inhibition. (Circulation Research 1991;69:277–291)

Recent studies from our laboratory have delineated important differences between the action potential characteristics of canine ventricular endocardium and epicardium.1 Chief among these is the manifestation of a spike and dome morphology in transmembrane action potentials recorded from ventricular epicardium, which is largely absent in endocardium. The presence of a prominent transient outward current ($I_{\text{to}}$) in epicardium but not in endocardium was shown to underlie these differences between the two tissue types. The presence of a spike and dome action potential morphology in epicardium but not in endocardium has been observed in canine, feline, and human hearts in vivo and in vitro using monophasic action potential or microelectrode recordings.2–6 Studies using ventricular myocytes have presented further
evidence in support of the hypothesis that these differences between epicardium and endocardium are due to the presence of an $I_n$ more prominent in epicardium than in endocardium.7–9

Recent work10–14 also indicates that the prominent presence of $I_n$ in epicardium but not in endocardium contributes to differences in the time and rate dependence of action potential duration (APD) and refractoriness in the two tissue types as well as to the rate-dependent changes in the T wave and J (Osborn) wave in the electrocardiogram; the presence of $I_n$ also contributes to the greater sensitivity of epicardium to ischemia and to the differential effects of various pharmacological agents on epicardium and endocardium.

Although the electrophysiological actions of sodium channel block have been well characterized in Purkinje and endocardial preparations,15–20 in vitro investigations of the actions of sodium blockers in epicardium are limited.12 The present study was designed to contrast the electrophysiological actions of sodium channel blockade in canine ventricular epicardium and endocardium and to test the hypothesis that the marked differences in the response of the two tissues to sodium channel block is, in part, due to the presence of an $I_n$ more prominent in epicardium than in endocardium.

Materials and Methods

Papillary muscles, right ventricular trabeculae, and right ventricular epicardial strips (−2.0×1.5×0.2 cm) were isolated from hearts removed from anesthetized (30 mg/kg body wt sodium pentobarbital) mongrel dogs of either sex (18–26 kg). The epicardial preparations were obtained by razor blade shavings (Dermatome power handle 3293 with cutting head 3295, Davol Simon, Cranston, R.I.) made parallel to the fiber orientation in the right ventricular free wall. Because we found no major differences between the characteristics of papillary muscles and trabeculae, we grouped these together in the presentation of results. No significant differences could be discerned between the activity of intact papillary muscles and that of strips shaved from the surface of these muscles. The terms endocardial and epicardial in this report refer to the muscle 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 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 (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 ranging from 200 to 2,000 msec using rectangular stimuli (1–3 msec, 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 with the use of glass microelectrodes filled with 2.7 M KCl (10–20-MΩ direct current resistance) connected to a high input–impedance amplification system (World Precision Instruments, Sarasota, Fla.). Amplified signals were displayed on an oscilloscope (Tektronix, Beaverton, Ore.) and photographed on a 35-mm kymographic camera (Grass Instrument Co., Quincy, Mass.) or recorded on FM tape (A.R. Vetter Co., Rebersburg, Pa.). The maximal rate of rise of the action potential upstroke (dV/dt$_{max}$ or V$_{max}$) was measured with a differentiator adjusted for linearity within the range of 50–500 V/sec. APD was measured as the interval between the upstroke and 90% repolarization of the action potential. Care was taken to avoid transitional cells in obtaining data representative of ventricular endocardium. In the case of papillary muscles, recordings were always made from the apical region, known to be devoid of Purkinje fibers.

Experiments were not started until the preparations were fully recovered and displaying stable electrophysiological characteristics. In the case of the epicardial sheets, this sometimes took ≥3 hours; the spike and dome morphology of the epicardial action potential was usually much attenuated when the tissue was first introduced into the bath and recovered slowly as the tissue hyperpolarized (washout of residual catecholamines leaking out of sympathetic nerve endings may have contributed to this).

Restitution of action potential variables (i.e., progressive changes in the action potential characteristics of premature beats as they are introduced progressively later in diastole) was determined with the use of a single test pulse (S$_2$) delivered after every fifteenth basic beat (S$_1$). The S$_1$–S$_2$ coupling interval was increased progressively from the end of the refractory period until the next basic beat. The effective refractory period (ERP) was defined as the longest S$_1$–S$_2$ interval at which S$_2$ failed to elicit a propagated response.

Drugs

4-Aminopyridine (Sigma Chemical Co., St. Louis) was dissolved in distilled water and made soluble by warming to yield a stock solution of 0.5 M. The pH of the stock was adjusted to 7.4 with HCl. Because 4-aminopyridine has been reported to cause release of neurotransmitters from adrenergic and cholinergic nerve endings,21 the combination of propanolol (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 when it was determined that they did not alter the actions of 4-aminopyridine. Ryandine (Merck Sharp & Dohme, Rahway, N.J.) was prepared as a stock solution of 1.0 mM. Tetrodotoxin (TTX, Calbiochem Corp., La Jolla, Calif.) was used in concentrations of 2–3 µM; flecainide (3 µM) was used in concentrations of 10–15 µM. A stock solution of 1 mg/ml DL-propranolol hydrochloride (Sigma Chemical) was prepared and
diluted with Tyrode’s solution to a final concentration of 1–5 μg/ml.

Statistics

Statistical analysis was performed using Student’s t test for paired or unpaired data, as indicated, analysis of variance, and Scheffe’s test, and linear and nonlinear regression techniques (ASYSTANT, Macmillan Software Co., New York).

Results

DL-Propranolol, in addition to its β-adrenergic blocking actions, is known to exert significant local anesthetic effects to block the sodium channels and thus to decrease the Vmax, amplitude, and duration of the action potential in Purkinje fibers and myocardial tissues.17,22

These characteristic effects of propranolol are illustrated in Figure 1, Panel A shows a progressive decrease in Vmax amplitude, and duration of action potentials recorded from a papillary muscle preparation during a 4-hour period of exposure to DL-propranolol (1 μg/ml). In contrast to the abbreviation of APD in endocardium, propranolol produced a marked prolongation of APD in epicardium during the first 3 hours of exposure to the drug (Figure 1B). Attending the progressive development of APD prolongation was an augmentation in the amplitude of the peak plateau, an increase in the delay between the upstrokes of phase 0 and phase 2, a diminution of the amplitudes of phase 0 and phase 1, and a progressive decrease in Vmax (bottom tracing). After 4 hours of exposure to propranolol, a further decrease in the amplitude of phase 1 of the epicardial action potential results in an all-or-none repolarization and loss of the action potential plateau or dome. The premature repolarization of the action potential at the end of phase 1 results in a marked abbreviation of the epicardial response.

In both epicardium and endocardium, the propranolol-induced electrophysiological changes developed gradually over a period of several hours. The time course for development of the propranolol-induced changes in phase 0 amplitude and APD for the preparations pictured in Figure 1 are graphically illustrated in Figure 2. In endocardium, APD changes paralleled those of phase 0 amplitude (Figure 2A). In epicardium, however, the progressive decrease of phase 0 amplitude was accompanied by a progressive increase of APD, until a further sharp decline in phase 0 amplitude during the fourth hour of exposure to the drug caused an abrupt abbreviation of APD (Figure 2B). With both epicardium and endocardium, a steady-state effect of propranolol was not achieved even after 4.5 hours of exposure to the drug. The results shown are from an experiment in which the same impalement was maintained throughout the experimental protocol. Similar results were obtained in five other experiments: APD of epicardium increased an average of 34.4±11.3 msec (p<0.01), whereas that of endocardium decreased by 23.8±5.6 msec (p<0.01) after 4 hours of exposure to 1 μg/ml DL-propranolol (basic cycle length [BCL]=1,000 msec). In two experiments, washout of propranolol was found to reverse the drug-induced electrophysiological changes very slowly, with a time constant in the order of hours. Although we did not systematically evaluate the time course of development of propranolol’s effects at different concentrations of the drug, we did observe that the time constant for onset of drug effects was abbreviated when higher concentrations of the drug were used (3–5 μg/ml).

A quantitative assessment of propranolol-induced changes in action potential parameters of epicardium and endocardium is presented in Table 1 (3 μg/ml, 30 minutes, eight experiments). As a control to these
and subsequent experiments, we studied five epicardial and endocardial preparations for a period of 4–7.5 hours in the absence of any drug or intervention. No statistically significant changes were noted in any of the action potential parameters.

To explain the paradoxical prolongation of APD in epicardium, we considered the hypothesis that propranolol-induced inhibition of sodium inward current (I_{Na}) causes a diminution of phase 0 amplitude, which then leads to a cascade of events in which the activation states and kinetics of several other ionic currents are altered. One possible scenario is as follows: drug-induced block of the sodium channels would cause I_{Na} and the calcium current (I_{Ca}) activated during phase 0 to be overwhelmed by I_{INa}, resulting in initiation of early repolarization (phase 1) at less positive potentials. Because phase 1 begins at less positive potentials and because the contribution of net inward current is weaker, phase 1 would proceed to more negative potentials. At these negative voltages, the availability of I_{Ca} would be considerably compromised because of inactivation and slower activation and reactivation kinetics. Because the net inward current available at the end of phase 1 (as I_{INa} inactivates) is diminished, the second upstroke giving rise to phase 2 (plateau) of the action potential should be slowed. Activation of the delayed rectifier (I_{K}) may likewise be delayed, thus permitting phase 2 to achieve a more positive potential before I_{K} overtakes I_{Cat} giving way to final repolarization. The slower second upstroke and the more positive voltage achieved during phase 2 would result in a delay of the start of phase 3, thus causing a prolongation of APD (Figure 1B).

With greater inhibition of I_{Na}, the relative influence of I_{INa} would be greater, resulting in termination of phases 0 and 1 at still more negative potentials. Net outward current during phase 1 may be so strong as to overwhelm I_{Ca} and any slowly inactivating sodium current (or sodium window current), thus causing an all-or-none repolarization. The failure of these inward currents to overtake the outward currents (mainly I_{INa} and I_{K}), as they normally do at the end of phase 1, would result in abolition of the dome and a marked abbreviation of APD.
As an initial test of this hypothesis, we examined the effects of TTX (2–3 μM), a selective blocker of the sodium channels. The effects of TTX to diminish Vmax phase 0 amplitude, and APD in Purkinje fibers and muscle preparations is well known.\(^{15,16,18,20,23}\) These actions of TTX are illustrated in Figure 3. TTX (3 μM) diminished Vmax and phase 0 amplitude in both epicardium and endocardium. These changes were accompanied by an abbreviation of APD in endocardium but a prolongation of APD in epicardium. With longer exposure of epicardium to TTX, the dome was abolished, resulting in a marked abbreviation of APD. In five experiments, APD of epicardium was prolonged an average of 11.1±6.3 msec (p<0.05), whereas the APD of endocardium was abbreviated by 10.8±12.9 msec (p<0.05) after 15 minutes of exposure to 2 μM TTX (BCL=1,000 msec).

The effects of TTX and their similarity to those of propranolol (compare Figures 1 and 3) provide support for the hypothesis that a paradoxical prolongation of APD in epicardium can result as a consequence of a decrease of phase 0 brought about by inhibition of \(I_{Na}\).

As a further test of the hypothesis, we examined the correlation between concurrent changes in different phases of the epicardial action potential during the progressive development of APD prolongation by exposure to propranolol. Figure 4 illustrates the results of an experiment in which data were recorded during a 4-hour period of exposure to 1 μg/ml propranolol. Panel A shows a significant correlation between the amplitudes of phase 0 and phase 1. A 0.64-mV change in the amplitude of phase 1 occurred for each 1-mV change in the amplitude of phase 0. Panels B, C, and D indicate a strong

**TABLE 1. Effect of DL-Propranolol on Action Potential Parameters in Canine Ventricular Epicardium and Endocardium**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PROP</th>
<th>Difference</th>
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<tbody>
<tr>
<td><strong>Epicardium</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Resting membrane potential (mV)</td>
<td>80.3±2.9</td>
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<td>0.7±4.0</td>
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<td>Amplitude (mV)</td>
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<tr>
<td>Phase 0</td>
<td>92.1±8.6</td>
<td>83.9±8.2</td>
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<tr>
<td>Phase 1</td>
<td>65.7±9.3</td>
<td>61.0±6.1</td>
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<td>Phase 2</td>
<td>87.5±5.5</td>
<td>99.3±5.4</td>
<td>11.8±6.7*</td>
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<td>Vmmax (mV)</td>
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<td></td>
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<tr>
<td>Time to peak plateau (msec)</td>
<td>43.3±9.2</td>
<td>60.0±8.4</td>
<td>16.7±10.7*</td>
</tr>
<tr>
<td>APD50 (msec)</td>
<td>133.5±9.7</td>
<td>155.2±8.9</td>
<td>21.7±9.3*</td>
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<tr>
<td>APD90 (msec)</td>
<td>154.3±15.2</td>
<td>179.8±17.5</td>
<td>25.5±12.8*</td>
</tr>
<tr>
<td>Vm (V/sec)</td>
<td>205.0±35.2</td>
<td>153.2±12.6</td>
<td>-51.8±46.5†</td>
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<td><strong>Endocardium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting membrane potential (mV)</td>
<td>82.1±0.8</td>
<td>81.2±1.6</td>
<td>-1.0±0.9</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td></td>
<td></td>
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<tr>
<td>Phase 0</td>
<td>111.3±4.1</td>
<td>100.0±2.3</td>
<td>-11.3±2.2*</td>
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<tr>
<td>Phase 1</td>
<td>99.8±1.7</td>
<td>99.0±2.2</td>
<td>-0.8±1.7</td>
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<tr>
<td>APD50 (msec)</td>
<td>144.5±4.3</td>
<td>130.5±5.4</td>
<td>-14.0±5.6*</td>
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<tr>
<td>APD90 (msec)</td>
<td>172.5±4.8</td>
<td>161.6±7.2</td>
<td>-10.9±6.9†</td>
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<tr>
<td>Vmmax (V/sec)</td>
<td>268.3±48.0</td>
<td>187.5±37.7</td>
<td>-80.8±59.8†</td>
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Values are mean±SD (n=8). PROP, 3 μg/ml propranolol; APD50 and APD90, action potential durations measured at 50% and 90% repolarization, respectively. Basic cycle length was 500 msec.

\(\ast p<0.01\) and \(\dagger p<0.05\) control vs. PROP by \(t\) test for paired data.

*FIGURE 3. Effect of tetrodotoxin (TTX) in isolated canine ventricular endocardium (panel A) and epicardium (panel B). Transmembrane recordings (top) and \(V_{m}\) tracings (bottom) were obtained before (solid line) and after (dotted line) exposure to 3 μM TTX. TTX decreased the amplitude and rate of rise of phase 0 in both the tissues. In endocardium, TTX abbreviated the action potential duration, whereas in epicardium, the decrease in phase 0 amplitude and phase 1 amplitudes resulted in a delayed second upstroke and an increase in action potential duration. A more negative termination of phase 1, after longer exposure to TTX, caused abolition of the epicardial plateau and marked abbreviation of the action potential. Basic cycle length was 300 msec.
correlation between the decrease in phase 1 amplitude and the concomitant changes in phase 2 and APD. These findings are concordant with our previous studies\(^{14}\) showing a significant correlation between phase 1 amplitude and time to peak plateau, plateau height (phase 2 amplitude), and APD in epicardium as a function of changes in the rate or prematurity of stimulation.

These remarkable effects of sodium channel block on epicardium, although of great interest, could be argued to be of little practical significance, since neither TTX nor such high concentrations of plasma propranolol are commonly encountered in the clinic. Recent years, however, have seen the advent of a new class of drugs capable of producing profound use-dependent block of the sodium channels at therapeutic levels. Among the drugs displaying this class IC action is flecainide.\(^{24,25}\) Therefore, it was of interest to determine whether the effect of flecainide (10–15 \(\mu\)M) in epicardium was similar to that of the other sodium channel blockers. Figure 5 shows the response of epicardium to 30 and 40 minutes of exposure to 15 \(\mu\)M flecainide. Flecainide produced changes very similar to those observed with DL-propranolol and TTX. After 30 minutes of flecainide,
V\textsubscript{m}, phase 0, phase 1, and phase 2 amplitudes of the epicardial action potential were diminished, and the time to peak plateau and APD increased. A further drop in the amplitude of phase 1 (after 40 minutes of flecainide) resulted in loss of the dome, resulting in a marked abbreviation of the epicardial response.

A more complete presentation of flecainide’s effects on action potential parameters in epicardium is provided in Table 2 (n=8). Flecainide-induced changes of the epicardial action potential were similar to those produced by propranolol in all respects but one. Unlike propranolol, flecainide did not give rise to a more positive peak plateau. Flecainide generally produced a slowing of the second action potential upstroke and a more negative peak plateau, changes suggestive of a weak inhibition of calcium current.

To explain the differential effects of sodium channel block on epicardium and endocardium, we considered the hypothesis that differences in the responsiveness of the two tissues to the drug were, in large part, due to the presence of an I\textsubscript{Na}\textsubscript{M}-mediated spike and dome (notch) in epicardium but not in endocardium. As an initial test of this hypothesis, we examined the rate dependence of action potentials in epicardium and endocardium in the presence of propranolol or TTX (Figures 6–8). Figure 6 illustrates the effects of an abrupt acceleration (panel A) and deceleration (panel B) on transmembrane activity in epicardium and endocardium after 20 minutes of exposure to DL-propranolol (3 μg/ml). In endocardium, deceleration resulted in an increase in the amplitude and duration of the action potential, and acceleration produced the opposite effects. Under the same con-

| Table 2. Effect of Flecainide on Action Potential Parameters in Canine Ventricular Epicardium |
|---------------------------------|-----------------|-----------------|
|                                | Control         | FLEC            | Difference     |
| Amplitude (mV)                 |                |                 |                |
| Phase 0                        | 91.1±6.7        | 81.9±8.1        | -9.3±6.6*      |
| Phase 1                        | 63.6±3.4        | 59.1±4.9        | -4.5±5.2†      |
| Phase 2                        | 101.0±3.9       | 98.4±7.7        | -2.6±6.2       |
| Time to peak plateau (msec)    | 49.4±8.8        | 65.1±9.1        | 15.8±8.9*      |
| APD\textsubscript{90} (msec)    | 148.7±12.7      | 164.1±10.6      | 15.4±7.5*      |
| APD\textsubscript{90} (msec)    | 168.6±15.0      | 188.6±12.5      | 19.9±8.2*      |
| V\textsubscript{m} (V/sec)      | 240.1±43.6      | 172.6±60.0      | -67.5±30.3*    |

Values are mean±SD (n=8). FLEC, 15 μM flecainide; APD\textsubscript{90} and APD\textsubscript{90} action potential durations measured at 50% and 90% repolarization, respectively. Basic cycle length was 500 msec.

*p<0.01 and †p<0.05 control vs. FLEC by t test for paired data.

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**Figure 6. Effects of abrupt acceleration and deceleration on the action potential characteristics of endocardium (Endo) and epicardium (Epi) in the presence of 3 μg/ml DL-propranolol.**

Panel A: Acceleration. Tracings depict the last action potential recorded at a basic cycle length (BCL) of 2,000 msec and the first five beats after a change to a BCL of 500 msec. Panel B: Deceleration. Action potentials shown represent the last beat recorded at a BCL of 300 msec and the first four beats at a BCL of 2,000 msec.
acceleration results in a transient appearance of the plateau. The gradual development of sodium channel block (use dependent) at the shorter BCL is likely responsible for the subsequent abolition of the dome.

When drug-induced block of the sodium channel was less intense, the dome of the action potential was not lost at physiological rates of stimulation. Under these conditions, acceleration of basic drive resulted in a progressively larger drug-induced prolongation of the APD at progressively shorter BCLs, as illustrated for propranolol in Figure 8. In this example, exposure of the epicardial preparation to a β-adrenergic blocking concentration of dl-propranolol (0.2 μg/ml) produced a slight upward shift of the APD–rate relation. Increasing the concentration to 3 μg/ml resulted in a larger upward shift and a flattening of the relation. The prolongation of APD after 3 μg/ml dl-propranolol was more pronounced at the faster frequencies (possibly because of the use dependence of propranolol’s local anesthetic effects). The composite results of eight similar experiments are presented in Figure 8B: propranolol (3 μg/ml) prolonged APD by 14.7% at a BCL of 2,000 msec and by 23.3% at a BCL of 300 msec.

Figure 9 illustrates restitution characteristics of transmembrane responses recorded from endocardium and epicardium after 270 minutes of exposure to propranolol (1 μg/ml, BCL=2,000 msec). The first beat in each panel is the last of a train of 15 basic beats. Subsequent beats represent premature responses introduced progressively later in diastole, once after every fifteenth basic beat. The restitution characteristics in endocardium are unremarkable. In epicardium, early premature beats show a restoration of the action potential dome, which is lacking in the basic beats and responses elicited later in diastole. Once again, the restoration of the dome is attended by an increase in the amplitude of phase 1 that is consistent with a decreased contribution of Ito. As Ito reactivates, phase 1 becomes progressively more prominent until it brings the membrane potential to a voltage at which the dome fails to appear. Figure 10 shows similar restitution characteristics recorded from epicardium after exposure to TTX (2 μM). Figure 10B illustrates the recovery of Vmax. Restoration of the action potential dome occurs at a time when Vmax is at its lowest level and is once more abolished when Vmax is largely recovered. Also noteworthy is the progressive decline in the phase 0 amplitude during the first three premature beats at a time when Vmax is increasing. These data suggest that restitution of action potential characteristics in epicardium is importantly influenced by the recovery of Ito and provide further support for the hypothesis that the differences in the responsiveness of the two tissues to sodium channel block are due, in large part, to the presence of a prominent Ito in epicardium but not in endocardium.

As a further test of this hypothesis, we examined the effects of propranolol and TTX in preparations pretreated with 4-aminopyridine, an Ito blocking...
agent. In the absence of 4-aminopyridine, TTX abbreviated APD in endocardium but prolonged it in epicardium (Figures 11A and 11B). In the presence of 4-aminopyridine (2 mM), the spike and dome morphology of the epicardial response was largely abolished so that it now more closely resembled that of endocardium. Under these conditions, TTX abbreviated APD in epicardium and decreased the amplitude of phase 0. Similar results were obtained in three other experiments in which the preparations were exposed to TTX.

In addition to a selective depression of APD in epicardium, TTX, propranolol, and flecainide were found to depress excitability in epicardium to a greater degree than in endocardium. Sodium blockade induced postrepolarization refractoriness, tachycardia-dependent activation delays (which often exhibited a Wenckebach periodicity), and conduction block in epicardium at a time when endocardium was largely unaffected.

Postrepolarization refractoriness and the development of long activation delays and block at faster frequencies were seen consistently in epicardium but not in endocardium after exposure to each of the three sodium channel blockers studied. This effect, coupled with the APD-prolonging effect of sodium blockade, served to flatten or reverse the relation between the ERP and stimulation rate in epicardium, as illustrated in Figure 12. Panels A and B show the effects of propranolol (3 μg/ml, 40 minutes) on the rate dependence of APD and ERP in epicardium and endocardium. ERP in this experiment was determined using S2 pulses with an intensity two and a half times diastolic threshold. In endocardium (Figure 12A), the propranolol-induced changes were unremarkable and consisted of a decrease in APD and ERP at all frequencies, producing a downward shift of the curves. In epicardium, however, propranolol produced an upward shift and a flattening of the APD-rate relation and a reversal of the ERP-rate

**Figure 8.** Rate dependence of action potential duration in epicardium in the absence (control) and presence of low (0.2 μg/ml, β-blocking) and high (3.0 μg/ml, sodium channel-blocking) concentrations of propranolol. Panel A: Action potential duration measured at 90% repolarization (APD90) plotted as a function of the basic cycle length (BCL). Panel B: Propranolol-induced prolongation of APD90, expressed as a percentage of control APD90, plotted as a function of BCL. Each point represents the mean ± SEM of eight experiments.
relation characterized by a marked increase in ERP at the faster rates of stimulation. At a BCL of 500, APD increased by about 15 msec, whereas ERP increased from 166 to 378 msec. ERP and APD could not be determined at BCLs shorter than 500 msec because of the loss of 1:1 activation at these rates.

Figure 12C shows results obtained from another epicardial preparation before and after similar treatment (3 μg/ml propranolol, 45 minutes) but using test pulses of four times diastolic threshold intensity. Propranolol produced a flattening of both the APD–ERP–rate relations.

The effects described above likely contribute importantly to the antiarrhythmic actions of sodium blockers but may also be responsible for some of the arrhythmogenic or proarrhythmic actions of these agents. An example of arrhythmogenic activity precipitated by a heterogeneous response to sodium blockade is illustrated in Figure 13.

Figure 13 shows transmembrane recordings from two sites in an epicardial sheet obtained after 40 minutes of exposure to flecainide (15 μM). At a BCL of 1,000 msec, the action potential dome was lost at both sites. A premature stimulus introduced at an S1–S2 interval of 145 msec elicited a response devoid of a dome at the proximal site but a response in which the dome was fully restored at the distal site. As a consequence of the large difference in repolarization times at the two sites, and perhaps other factors, a reentrant beat appeared at the proximal site (third beat in bottom tracing). In other experiments, induction of epicardial reentry after exposure to flecainide could be readily achieved after either premature stimulation or after acceleration of the stimulation rate. Although the precise mechanism for the reentrant activity cannot be discerned from the available data, the results suggest that heterogeneity in the response of “normal” epicardium to sodium block can readily set the stage for reentry and other arrhythmias.

Discussion

Class I antiarrhythmic agents are among the most widely used of antiarrhythmic drugs. Although grouped into three subclasses, all possess the ability to block the sodium channels in cardiac tissues. Sodium channel block is a feature common to many other antiarrhythmics as well. The electrophysiological effects of sodium channel block have generally been thought to be quite uniform, straightforward, and, therefore, well understood. They generally consist of 1) suppression of the rate of rise (V_max) and the amplitude of phase 0 of the action potential via inhibition of the fast I_{Na}, and 2) abbreviation of APD secondary to block of the sodium “window currents” or slowly inactivating TTX-sensitive sodium current.15,20,23 Our data demonstrate for the first time an effect of sodium channel block to prolong APD in ventricular myocardium in vitro. The results point to a heterogeneous and, in some cases, opposite response of epicardial and endocardial ventricular tissues to sodium channel block and delineate a novel mechanism by which flecainide and other sodium channel blockers may produce proarrhythmic effects. Notably, none of the three agents significantly affected I_{Na}.

Previous findings10 have suggested the existence of a marked heterogeneity of active membrane properties in muscle cells spanning the ventricular wall, which may have far-reaching implications with re-
spect to our understanding of cardiac electrophysiology, electrocardiography, pathophysiology, and pharmacology. The presence of a prominent \( I_{\text{Ca}} \)-mediated spike and dome in action potentials recorded from epicardium but not from endocardium is thought to contribute to the selective depression of epicardium during ischemia.\(^{10,26} \) The manifestation of an Osborn or J wave in the electrocardiogram,\(^{3} \) differences in the rate dependence of APD and refractoriness in the two tissue types, and differences in the sensitivity of these two tissues to \( K^+ \), \( Ca^{2+} \), and a variety of pharmacological agents including quinidine, 4-aminopyridine, ryanodine, calcium blockers, amiloride, acetylcholine, and isoproterenol,\(^{1,10,11,13,27} \) The present study demonstrates similar differences in the responsiveness of epicardium and endocardium to sodium channel block.

Sodium channel block was achieved in this study with either TTX, DL-propranolol, or flecainide. TTX is a highly specific blocker of the sodium channel and is known to block the cardiac sodium channel in a use-dependent manner.\(^{28,29} \) After TTX exposure, the amplitude and \( V_{\text{max}} \) of phase 0 reach a steady state fairly quickly; this occurrence depends principally on equilibration of TTX with the extracellular space (time constant of \( \sim 10 \) minutes in canine epicardium).\(^{30} \) Propranolol is a \( \beta \)-adrenergic blocker with reasonably specific actions: cardiac sodium channels are blocked at concentrations of 0.2–3 \( \mu \)g/mL. Blocking of the sodium channel by propranolol is known to develop very slowly over a period of hours and to be linked to the slow tissue uptake (intracellular accumulation) of the drug.\(^{22} \) Our results show a slow onset of propranolol's electrophysiological actions in canine ventricular epicardium and endocardium as well as a slow washout of these effects, consistent with these earlier studies. The effects of TTX and of very high concentrations of propranolol, while of scientific interest, are not clinically relevant. Flecainide, on the other hand, is a Class IC antiarrhythmic agent capable of producing comparable inhibition of the sodium current at clinically relevant concentrations. Flecainide's predominant action is to block cardiac sodium channels. Reduction of \( V_{\text{max}} \) is very rate dependent (use dependent), because recovery from flecainide block of the sodium channels occurs very slowly.\(^{24,25} \)

With concentrations of the drug producing nearly comparable decreases in \( V_{\text{max}} \) propranolol produced an abbreviation of APD in endocardium that was approximately twice that produced by TTX (23.8±5.6 versus 10.8±12.9 msec) and a prolongation of APD in epicardium that was greater than threefold that produced by TTX (34.4±11.3 versus 11.1±6.3 msec) at a BCL of 1,000 msec. These differences may be due to a number of factors. Chief among these is the lack of specificity of propranolol as a sodium channel blocker; because it antagonizes any residual adrenergic tone (through spontaneous release of catecholamines from the adrenergic nerve endings), propranolol would be expected to exert a prolonging effect on APD and thus could accentuate the effects of sodium blockade in epicardium but antagonize them in endocardium (see Figure 8). The extent to which these factors influence our results awaits more detailed study. It is noteworthy that in a corollary study\(^{13} \) we have recently found that isoproterenol abbreviates APD to a greater extent in epicardium than in endocardium. Comparison of the effects of propranolol and flecainide under similar conditions shows that the two produce a more comparable prolongation of APD in epicardium (Tables 1 and 2), although the effect of propranolol was consistently greater than that of flecainide.

Our results appear to be at odds with those of Gilmour and Zipes,\(^{12} \) who showed that TTX abbreviates action potentials recorded from isolated canine endocardial as well as epicardial tissues. The reason for the discrepancy is not clear but may be due to several factors. Chief among these is the virtual absence of a spike and dome morphology in action potentials recorded from their epicardial preparations and the higher concentration of TTX (5 \( \mu \)M) used. The small notch may be related to their use of younger animals, since the spike and dome morphology is known to be absent in the neonate and to become progressively more prominent with age.\(^{31,32} \) or it may be related to a briefer equilibration of the tissues after isolation.

The prolongation of ventricular refractoriness (epicardial) by TTX and propranolol has previously
been demonstrated in in vivo studies. Gilmour et al.\(^ {133} \) demonstrated that intracoronary TTX increases epicardial refractoriness, whereas Kupersmith et al.\(^ {134} \) showed that administration of propranolol increases the epicardial refractory period and APD of the monophasic action potential in both normal and ischemic epicardium. In both instances bilateral stellectomy prevented these effects, suggesting that both were attributable to inhibition of sympathetic nervous system activity. A direct effect on epicardium was not seen and indeed would not be expected, since there was no evidence that the doses used produced much inhibition of the sodium current: little or no change in conduction velocity was observed in normal myocardium after either TTX or propranolol exposure.

The data presented in our study support the hypothesis that the paradoxical prolongation of APD in epicardium is due, in large part, to a decrease in the amplitude of phases 0 and 1 (secondary to sodium channel block), which, in turn, alters the intensity and kinetics of several ionic currents, causing subsequent phases of the epicardial action potential to be shifted with respect to time and voltage (Figures 1–4). In this schema, a decrease of inward current in the early phases of the action potential contributes to a diminution of phase 0 amplitude and termination of phase 1 at more negative potentials. The availability of \( I_{Ca} \) at these more negative potentials is diminished, thus causing a slowing or delay in the emergence of the second upstroke. The net inward current at the end of phase 1 may also be diminished because the sodium blockers inhibit the slowly inactivating and window sodium currents.\(^ {15,16,23} \) The delay in the development of the second upstroke is usually attended by an increase in the voltage of the plateau phase. A correlation between phase 1 amplitude and plateau height is also observed in epicardium after changes in the rate or prematurity of stimulation.\(^ {14} \) The reason for the increase in the height of the

**FIGURE 13.** Two transmembrane recordings showing flecainide-induced reentry in epicardium. The recordings were obtained from proximal (P) and distal (D) sites of an epicardial preparation exposed to flecainide (15 \( \mu \)M). The first beat in each tracing is the last of a train of 10 basic beats. A premature stimulus introduced at a coupling interval of 145 msec elicited a response devoid of a dome at the proximal site but a response in which the action potential dome was fully restored at the distal site. The large difference in repolarization times would be expected to give rise to a strong electrotonic current flow between the two sites. This local current operating through a circuit movement or reflection mechanism is likely responsible for the reentrant excitation of the proximal site (third beat in bottom tracing). Basic cycle length was 1,000 msec.
plateau is unclear but may be related to a delay or slowing of the activation kinetics of $I_K$ that is due to the changes in the activation of $I_{Ca}$ \cite{35}. In any case, final repolarization is delayed, resulting in a prolongation of APD.

Flecainide differs from TTX and propranolol in that accentuation of the spike and dome is not accompanied by an increase in the voltage of the peak plateau (possibly because of an effect of the drug on calcium currents at concentrations $>10 \mu M$\cite{36}).

Another major difference in the responsiveness of epicardium and endocardium to sodium channel block is the marked abbreviation observed in epicardium after abolition of the dome. The same basic mechanism outlined above may explain these actions of sodium blockers. When the termination of phase 1 shifts to potentials negative to the threshold voltage for activation of $I_{Ca}$, the outward currents would be expected to overwhelm any activated inward currents, resulting in an all-or-none repolarization and marked abbreviation of APD.

To explain the differential effects of sodium blockade on epicardium and endocardium, we considered the hypothesis that differences in the responsiveness of the two tissues to the drugs were, in large part, due to the presence of an $I_m$-mediated spike and dome (notch) in epicardium but not in endocardium. The rate and time dependence of these effects of sodium channel block in epicardium and endocardium (Figures 6–10) as well as the elimination of the differences in the responsiveness of the two tissues when pretreated with the $I_{to}$ blocker 4-aminopyridine (Figure 11) provide strong evidence in support of the hypothesis.

The differential effects of sodium blockade on the excitability or responsiveness of the two tissue types, although somewhat tangential to the scope of the present study, are included for completeness. The development of postpolarization refractoriness in epicardium but not in endocardium was a consistent finding (Figure 12). This effect of sodium blockade may help explain recent reports of a differential effect of chronic amiodarone treatment on epicardial and endocardial tissues\cite{37,38}. Full characterization of these differences and elucidation of the underlying mechanism await further study.

**Physiological and Clinical Implications**

The ability of sodium channel blockade to prolong the action potential and to induce postpolarization refractoriness at rapid stimulation rates may represent important mechanisms for the suppression of ventricular arrhythmias, especially where epicardium is a critical part of the ventricular tissue responsible for either the genesis or maintenance of the arrhythmia. The ability of sodium blockers to flatten or reverse the APD–rate or ERP–rate relations in epicardium (Figures 7 and 12) but not in endocardium by causing a greater relative prolongation of APD and/or inducing postpolarization refractoriness in epicardium at rapid stimulation rates is a novel finding. Indeed, the augmentation by sodium channel blockers of refractoriness in epicardium (but not in endocardium) at faster rates of stimulation approaches the type of response one might expect from an “ideal” antiarrhythmic agent\cite{39}. It is noteworthy that quinidine, a class IA antiarrhythmic agent with sodium channel–blocking effects as well as $I_K$-inhibiting effects, does not exhibit this behavior in epicardium\cite{10}.

The dramatic increase in refractoriness with acceleration of the stimulation rate is seen when the intensity of the stimulus is relatively weak (2.5 times diastolic threshold, Figure 12). This type of behavior might be representative of the behavior expected when the intensity of the activating wave front is diminished because of underlying disease (e.g., complex anisotropy due to fibrosis or surviving islands of activity after infarction) or the behavior resulting from the fractionation of wave fronts like that occurring during ventricular fibrillation. When the intensity of the test stimulus is increased, the changes in ERP of epicardium parallel those of APD, resulting in a flattening of the ERP–rate relation (Figure 12C). This behavior may be more representative of the behavior expected in tissue activated by a normal wave front.

By the same token, sodium channel block in epicardium, when sufficiently great to cause loss of the action potential dome and marked abbreviation of APD, can dramatically increase the degree of dispersion of refractoriness and thus exert an arrhythmogenic or proarrhythmic effect (Figure 13). This type of dispersion has been demonstrated after simulated ischemia in canine ventricular epicardial and endocardial tissues\cite{26} and in response to acetylcholine\cite{13} and hypothermia\cite{10}. The resulting heterogeneity provides an ideal substrate for the development of reentry. Our data suggest that a nonuniform response to sodium channel blockade can contribute to the development of a large dispersion of refractoriness in normal ventricular tissues and that this effect may be intensified under conditions of ischemia.

The presence of a prominent spike and dome morphology in M cells in the deep subepicardial layers of the canine ventricle\cite{40} raises the possibility that similar effects of sodium block may occur in more than just the epicardial surface.

The dispersion of APD in response to sodium channel blockade may occur between epicardium and endocardium (Figures 1, 3, 6, and 7) as well as between neighboring epicardial sites (Figure 13). A greater dispersion between epicardium and endocardium may be expected with flecainide than with either TTX or propranolol, because loss of the dome in epicardium is accompanied by an abbreviation of APD in endocardium after exposure to TTX and propranolol, whereas a prolongation of APD in endocardium occurs after exposure to flecainide\cite{36} because of its inhibition of $I_K$\cite{41}. 

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Acceleration-induced loss of the dome is observed more readily with flecainide than with TTX or propranolol, possibly because of its more accentuated use-dependent block of the sodium channels (acceleration decreases I_{Na} more than it reduces I_{Ca}). A weak inhibition of I_{Ca} by flecainide may also contribute to this effect. The tachycardia dependence of this potentially arrhythmogenic effect of flecainide is consistent with clinical reports that indicate that the proarrhythmic effects of the drug are generally exercise-induced and are related to the drug-induced slowing of ventricular conduction, as evidenced by rate-dependent increases in QRS duration.42–44

Acknowledgments

We wish to thank Judy Hefferson and Robert Goodrow for their skilled technical assistance. We are grateful to Dr. Di Diego for assistance with some of the experiments.

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**Key Words**: sodium channel block, ventricular myocardium, epicardium, endocardium, electrophysiology, tetrodotoxin, propranolol, flecainide
Sodium channel block produces opposite electrophysiological effects in canine ventricular epicardium and endocardium.
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doi: 10.1161/01.RES.69.2.277

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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