JACC Vol. 14, No. 4
October 1989:1053–66

Rate Dependence of Action Potential Duration and Refractoriness in Canine Ventricular Endocardium Differs From That of Epicardium: Role of the Transient Outward Current

SILVIO H. LITOVSKY, MD, CHARLES ANTZELEVITCH, PHD

Utica, New York

Previous studies have provided evidence for an important contribution of the transient outward current to the electrical activity of canine ventricular epicardium, but not endocardium. The present study examines the characteristics of action potential duration and refractoriness in these two tissue types. The time and rate dependence of changes in action potential duration and refractoriness observed in epicardium were significantly more accentuated than in endocardium. The restitution of action potential duration in epicardium paralleled the restitution of phase 1 amplitude of the action potential in this tissue. The correlation between phase 1 amplitude and action potential duration recorded from a large number of epicardial and endocardial preparations was significant under both steady state and restitution conditions. 4-Aminopyridine, a transient outward current blocker, decreased the time dependence of phase 1 amplitude and concomitantly decreased the time dependence of action potential duration in epicardium. 4-Aminopyridine abbreviated the action potential duration of epicardium at slow stimulation rates but had little effect or prolonged it at fast rates or after premature stimulation. (The availability of a transient outward current is relatively small after premature stimulation.)

The data support the hypothesis that the prominent presence of a transient outward current in epicardium, but not endocardium, contributes to the differences in the time and rate dependence of action potential duration and refractoriness in the two tissue types. The results also demonstrate the effect of an outward current to *prolong* the action potential and the effect of an outward current blocker to *abbreviate* the action potential. These observations may also aid in our understanding of the basis for rate-dependent changes in the T wave and J wave of the electrocardiogram, the rate dependence of cardiac arrhythmias, the greater sensitivity of epicardium to ischemia and the differential effects of drugs on muscle tissues spanning the ventricular wall.

(J Am Coll Cardiol 1989;14:1053–66)

The rate dependence and restitution of action potential duration in ventricular myocardium have been the subjects of numerous studies (1–8), most of which have employed endocardial preparations to examine these variables. However, these findings have been generalized to the entire myocardial mass. Recent work from our laboratory (9) has delineated important differences between the action potential characteristics of canine ventricular endocardium and epicardium. Chief among these was the manifestation of a spike and dome configuration in transmembrane action potentials recorded from ventricular epicardium that was largely absent in endocardium. The presence of a prominent transient outward current in epicardium, but not in endocardium, appears to account for these and other differences between canine epicardium and endocardium.

The present study was designed to compare the time and rate dependence of action potential duration in the two tissue types and to test the hypothesis that these differences are, in large part, secondary to differences in the relative contribution of the transient outward current to the electrical activity of the respective tissues. The physiologic and clinical implications of these findings are discussed.

Methods

Experimental preparation. Papillary muscles, right ventricular trabeculae and right ventricular epicardial strips (approximately 2.0 × 1.5 × 0.2 cm) were isolated from hearts removed from anesthetized (sodium pentobarbital, 30

From the Masonic Medical Research Laboratory, Utica, New York. This study was supported by Grants HL07600 and HL37396 from the National Institutes of Health, Bethesda, Maryland and a grant from the American Heart Association, New York State Affiliate, Syracuse, New York.

Manuscript received November 15, 1988; revised February 7, 1989, accepted March 17, 1989.

Address for reprints: Charles Antzelevitch, PhD, Masonic Medical Research Laboratory, 2150 Bleecker Street, Utica, New York 13504.

0735-1097/89/$3.50

JACC Vol. 14, No. 4
October 1989:1053-66

mg/kg body weight) mongrel male dogs. The epicardial preparations were obtained by razor blade shavings (Davol Simon Dermatome Power Handle 3293 with cutting head 3295) made parallel to the fiber orientation in the right ventricular free wall. Because we found no major differences between the characteristics of the papillary muscles and those of trabeculae, we have grouped these together in the presentation of the results. It should be noted that no significant difference could be discerned between the activity of intact papillary muscles and that of strips shaved from the surface of these muscles (9). 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 h while superfused with an oxygenated (95% oxygen, 5% carbon dioxide) Tyrode's solution (37 ± 0.5° C; pH = 7.35). Unless otherwise indicated, the composition of Tyrode's solution was (in m*M*): sodium chloride (NaCl) 129, potassium chloride (KCl) 4, sodium phosphate (NaH_2PO_4) 0.9, sodium carbonate ($NaHCO_3$) 20, calcium chloride ($CaCl_2$) 1.8, magnesium sulfate ($MgSO_4$) 0.5 and D-Glucose 5.5.

Action potential recordings. The tissues were stimulated at basic cycle lengths ranging from 200 to 2,000 ms with use of rectangular stimuli (1 to 3 ms 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 with use of glass microelectrodes filled with 2.7 *M* KCl (10 to 20 MΩ direct current resistance) connected to a high input-impedance amplification system (WPI). Amplified signals were displayed on an oscilloscope (Tektronix) and photographed on a 35 mm kymographic camera (Grass) or recorded on FM tape (Vetter). The maximal rate of rise of the action potential upstroke (Vmax) was measured with a differentiator adjusted for linearity with the range of 50 to 500 V/s. The duration of the action potential was measured as the interval between the upstroke and 90% repolarization of the action potential (APD_{90}). 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, which is known to be devoid of Purkinje fibers.

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 use of single test pulses (S_2) delivered after every 10th basic beat (S_1). The S_1S_2 coupling interval was increased progressively from the end of the refractory period until the next basic beat. The effective refractory period was defined as the longest S_1S_2 interval at which S_2 failed to elicit a propagated response.

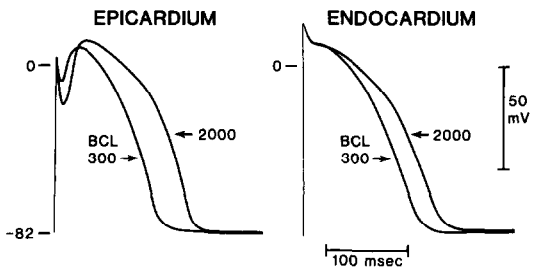


Figure 1. The effect of stimulation rate on transmembrane activity recorded from canine ventricular epicardial and endocardial preparations. Each panel shows superimposed traces of action potentials recorded at basic cycle lengths (BCL) of 300 and 2,000 ms. Epicardium, unlike endocardium, shows rate-dependent changes in the early phases of the action potential that contribute to the overall prolongation of the action potential after slowing of the stimulation rate.

Drug assessment. 4-Aminopyridine (Sigma) 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 hydrogen chloride. Because 4-aminopyridine has been reported to cause release of neurotransmitters from adrenergic and cholinergic nerve endings (10), 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 when it was determined that they did not alter the actions of 4-aminopyridine.

Statistical analysis. This was performed using the Student's *t* test for paired or unpaired data, as indicated, and linear and nonlinear regression techniques (Asystant).

Results

Rate dependence of action potential duration under steady state conditions. The presence of a transient outward current in canine ventricular epicardium contributes to a rapid repolarization of the epicardial action potential (phase 1) after the initial depolarization phase. The slow recovery of this current, once activated, has been shown to produce important rate-dependent changes in the early phases of the action potential (9). The lack of such changes in canine ventricular endocardium has been attributed to the absence of a prominent outward current in this tissue. Figure 1 illustrates these characteristics of endocardium and epicardium and shows that tissue-specific differences in the rate dependence of the early phases can, in turn, contribute to differences in the rate dependence of action potential duration.

In epicardium, a shift from a basic cycle length of 300 ms to 2,000 ms produces a marked accentuation of the spike and dome configuration of the action potential. Phase 1 becomes more prominent and the peak plateau is achieved later,

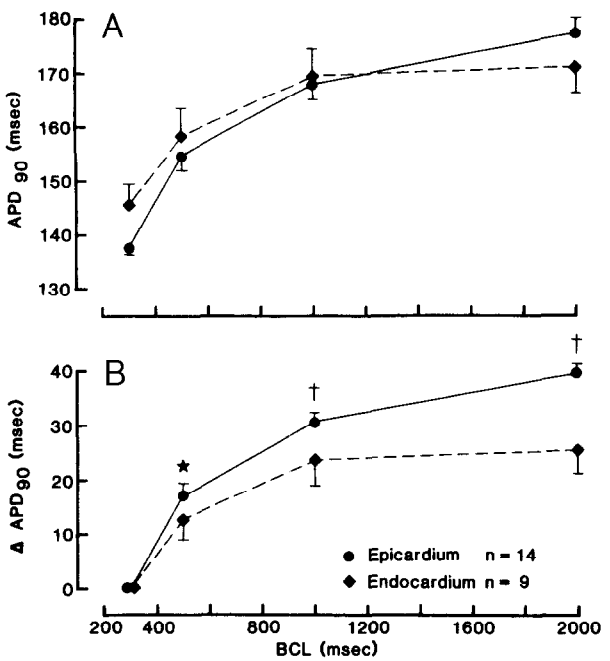


Figure 2. The action potential duration (APD)-rate relations of epicardium and endocardium. **A,** Action potential duration measured at 90% repolarization (APD_{90}) is plotted as a function of the basic cycle length (BCL) (steady state conditions; average of 14 epicardial and 9 endocardial preparations). **B,** Increase (Δ) in APD_{90} produced by prolongation of the basic cycle length (BCL) from 300 ms to 500, 1,000 and 2,000 ms. *p < 0.05, †p < 0.01. Significance determined by unpaired Student's *t* test for differences between epicardium and endocardium.

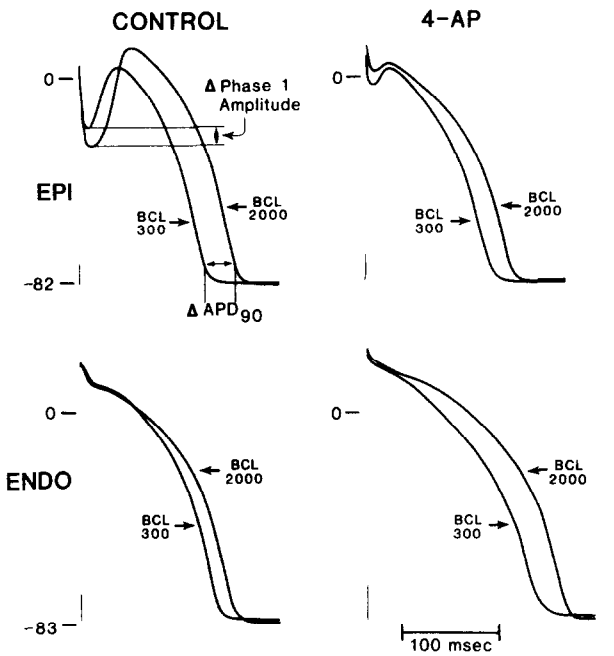


Figure 3. The effect of 4-aminopyridine (4-AP, 1 m*M*), a transient outward current blocking agent, on transmembrane activity recorded from epicardium (EPI) and endocardium (ENDO) compared with control. Each panel shows two superimposed traces recorded at a basic cycle length (BCL) of 300 and 2,000 ms. 4-Aminopyridine greatly diminished the spike and dome configuration of the epicardial response and reduced the deceleration-induced prolongation of the epicardial action potential. In endocardium, 4-aminopyridine had no effect on the early phases of the action potential and increased the rate-dependent changes in action potential duration (APD).

usually reaching a more positive potential. In contrast, no change is observed in the early phases of the action potential recorded from endocardium. The delay of the second upstroke of the epicardial response after deceleration can be seen to contribute to a greater prolongation of action potential duration in epicardium than in endocardium.

Figure 2 summarizes the results of 23 experiments in which a complete scan of stimulation frequencies was performed. At rapid stimulation rates, the epicardial action potential was briefer than that of endocardium; the converse was true at slow rates (Fig. 2A). The result was a crossover of the action potential duration rate relations at a basic cycle length between 900 and 1,200 ms. Whereas epicardium always showed a progressive prolongation of action potential duration at basic cycle lengths >1,000 ms, endocardium generally did not. In some endocardial preparations, we observed a small decrease in action potential duration as cycle length was increased from 1,000 to 2,000 ms. In four endocardial and four epicardial preparations we also evaluated the rate dependence of the effective refractory period; in all cases the changes paralleled those of action potential duration.

Figure 2B plots the deceleration-induced increase in action potential duration. Its rate dependence was clearly more pronounced in epicardium. Over the range of frequencies studied, APD prolonged an average of 38.7 ± 3.9 ms (28.4%) in epicardium and 25.6 ± 4.5 ms (17.5%) in endocardium ($p < 0.01$).

Effect of 4-aminopyridine on the action potential duration-rate relation. Because rate-dependent changes in the early phases of the epicardial action potential are believed to be due to the time dependence of reactivation of the outward currents and because these changes clearly contribute to the rate dependence of action potential duration in this tissue, we examined the effects of an outward current blocker, 4-aminopyridine, on the action potential duration rate relations (Fig. 3 and 4). 4-aminopyridine (1 m*M*) greatly attenuated the spike and dome configuration of the epicardial action potential, thereby diminishing the rate dependence of the time to peak plateau and the rate dependence of action potential duration (top panels, Fig. 3). In this example, the APD_{90} difference between responses obtained at a basic cycle length of 300 and 2,000 ms was decreased by 12 ms (delta APD_{90}: control = 34 ms, 4-aminopyridine = 22 ms). In contrast, 4-aminopyridine produced little change in the early

JACC Vol. 14, No. 4
October 1989:1053–66

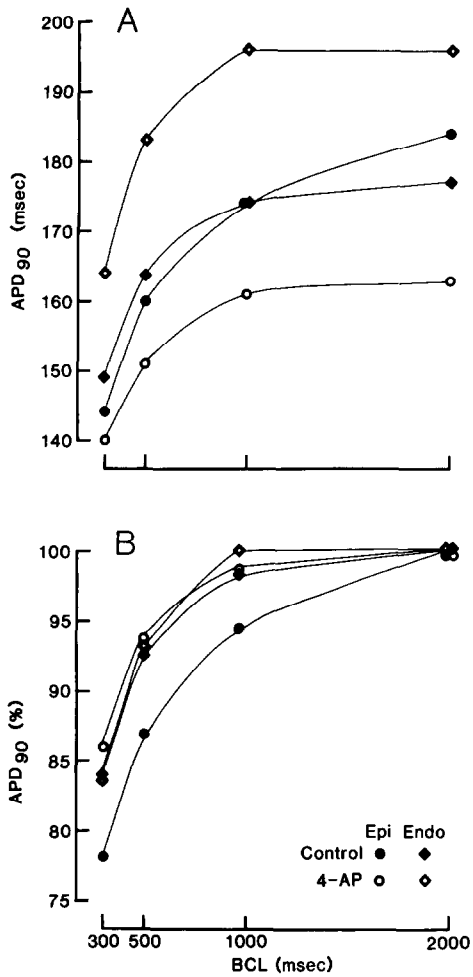


Figure 4. The effect of 4-aminopyridine (4-AP, 1 m*M*) on the action potential (APD)-rate relation in epicardium (Epi) and endocardium (Endo). **A,** The action potential duration measured at 90% repolarization (APD_{90}) is plotted as a function of stimulation basic cycle length (BCL). **B,** Percent change in action potential duration with acceleration of stimulation rate from a basic cycle length of 2,000 ms. 4-Aminopyridine produced little change in the relation recorded from endocardium, but there was a prominent change in the shape of the APD-rate relation of epicardium, making it similar to that of endocardium.

phases of the endocardial action potential and increased the APD_{90} difference between responses recorded at a basic cycle length of 300 and 2,000 ms (bottom panels, Fig. 3; delta APD_{90}: control = 19 ms, 4-AP = 30 ms).

It is noteworthy that after exposure to 4-aminopyridine the epicardial action potential displayed a more pronounced spike and dome at the faster stimulation rate, a situation opposite to that observed under control conditions. A possible explanation may be that this agent's block of the K^+ ion channels carrying the transient outward current may be voltage or pause dependent, as has been demonstrated in other tissues (11). On the basis of observations in nerve, unblocking of the channels would be expected at positive potentials attending each active response. This unblocking would lead to a relatively more intense outward current at faster stimulation rates despite a less complete reactivation of the current at these rates.

Figure 4A graphically illustrates the effect of 4-aminopyridine on the action potential duration-rate relations of epicardium and endocardium in similar experiments. In epicardium, this agent greatly abbreviated the action potential duration at long basic cycle lengths but exerted a negligible effect at a cycle length of 300 ms. Moreover, the increase in action potential duration between a basic cycle length of 1,000 and 2,000 ms present in the control study was nearly abolished after administration of 4-aminopyridine. In contrast, this agent prolonged the action potential duration of endocardium at all basic cycle lengths. The normalized plots (Fig. 4B) serve to illustrate that 4-aminopyridine produced little change in the shape of the action potential duration rate relation in endocardium, suggesting an action of the drug on a time-independent current, e.g., block of the inward rectifier, I_{K1}. However, the alteration in the shape of the relation in epicardium is consistent with an effect of this drug on a slowly reactivating time-dependent current, e.g., block of the transient outward current. Thus, 4-aminopyridine alters the configuration of the action potential (Fig. 3) and the action potential duration-rate relation (Fig. 4) of epicardium in such a way that both more closely resemble those of endocardium.

A summary of the effects of 4-aminopyridine on epicardial and endocardial action potential variables is presented in Table 1. In epicardium, the drug produced significant changes in all variables except the rest potential at a basic cycle length of 2,000 ms. At a basic cycle length of 300 ms, the drug effect on the early phases of the action potential was less pronounced, and the changes in action potential duration were opposite to those observed at a basic cycle length of 2,000 ms. In endocardium, 4-aminopyridine produced only minor changes in the early phases of the action potential; at a basic cycle length of 2,000 ms, it prolonged the action potential duration of endocardium but abbreviated that of epicardium; at a basic cycle length of 300 ms, it increased the action potential duration of both tissues.

Correlation between phase 1 amplitude and action potential duration. Because there is considerable variability in the phase 1 amplitude of action potentials recorded from different epicardial and endocardial preparations, we sought to determine whether a correlation exists between the rate dependence of phase 1 amplitude and the rate dependence of action potential duration. Figure 5 shows the results obtained from 23 epicardial and endocardial preparations. The difference in action potential duration of responses elicited at a basic cycle length of 2,000 and 300 ms (normalized for the value at a cycle length of 2,000 ms) is plotted as a function of the difference in phase 1 amplitude. The data show a significant correlation between the rate-dependent changes

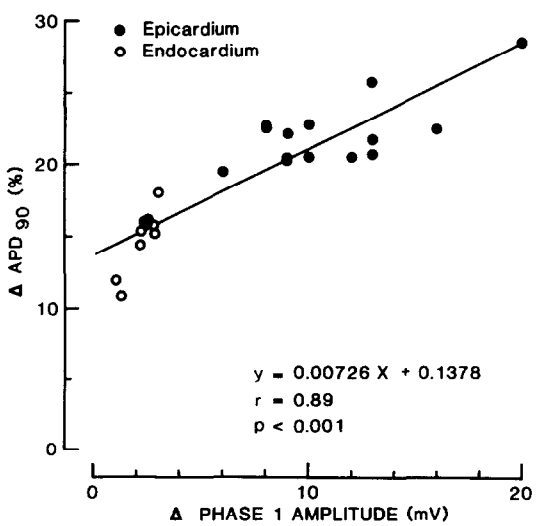
Table 1. Effect of 1 m*M* 4-Aminopyridine (4-AP) on Action Potential Parameters in Epicardium and Endocardium Under Steady State Stimulation Conditions

	BCL = 300 ms			BCL = 2,000 ms		
	Control	4-AP	Delta	Control	4-AP	Delta
Epicardium (n = 8)						
Rest potential (−mV)	81.3 ± 1.9	80.6 ± 1.7	−0.6 ± 0.5	81.5 ± 1.9	81.6 ± 1.5	0.1 ± 1.2
Amplitude						
Phase 0 (mV)	90.5 ± 8.0	95.3 ± 5.7	4.8 ± 4.1†	91.1 ± 7.9	97.1 ± 5.4	6.0 ± 4.6†
Phase 1 (mV)	72.1 ± 8.9	87.0 ± 5.3	14.9 ± 6.8†	64.9 ± 10.0	88.3 ± 4.4	23.4 ± 8.8†
Phase 2 (mV)	91.3 ± 6.4	91.6 ± 5.0	0.4 ± 4.2	96.5 ± 5.9	92.3 ± 4.7	−4.3 ± 5.1*
Phase 1 magnitude (mV)	18.4 ± 10.5	8.3 ± 5.2	−10.1 ± 6.1†	26.3 ± 11.4	8.9 ± 4.7	−17.4 ± 7.8†
APD_{50} (ms)	113.1 ± 6.8	113.3 ± 9.9	0.1 ± 9.7	151.4 ± 13.7	136.3 ± 13.7	−15.1 ± 15.6*
APD_{90} (ms)	137.3 ± 9.0	145.4 ± 12.0	8.1 ± 9.6	178.6 ± 13.4	170.0 ± 16.7	−8.6 ± 11.9*
Endocardium (n = 6)						
Rest potential (−mV)	81.0 ± 1.1	81.1 ± 1.5	0.2 ± 0.8	81.3 ± 1.2	81.8 ± 1.3	0.5 ± 0.5
Amplitude						
Phase 0 (mV)	103.7 ± 3.3	105.5 ± 4.4	1.8 ± 1.2†	104.2 ± 3.9	106.8 ± 4.6	2.7 ± 1.8†
Phase 2 (mV)	95.5 ± 3.8	97.2 ± 6.6	1.7 ± 3.5	95.2 ± 3.9	98.2 ± 6.6	3.0 ± 4.0
APD_{50} (ms)	113.1 ± 5.8	127.7 ± 11.6	14.5 ± 7.4†	138.5 ± 5.3	156.8 ± 10.1	18.3 ± 7.9†
APD_{90} (ms)	143.3 ± 9.7	160.7 ± 14.2	17.3 ± 9.6†	170.3 ± 9.6	193.0 ± 14.9	22.7 ± 11.3*

*p < 0.05; †p < 0.01. All values are given as mean values ± SD. APD_{50} and APD_{90} = action potential durations measured at 50% and 90% of full repolarization; BCL = basic cycle length; n = number of preparations.

in phase 1 amplitude and the rate-dependent changes in action potential duration. 4-Aminopyridine decreased the rate dependence of action potential duration coincidentally with a decrease in the rate dependence of phase 1 amplitude (not shown).

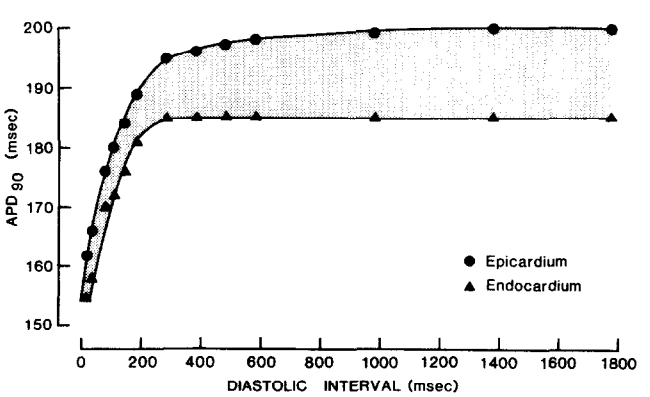
Figure 5. Relation between the rate dependence of action potential duration (APD) and phase 1 amplitude in epicardium and endocardium. The percent change in action potential duration produced by deceleration of the stimulation rate from a basic cycle length (BCL) of 300 ms to 2,000 ms is plotted as a function of the change in phase 1 amplitude of the same two responses (see Fig. 3). The data, obtained from 23 different preparations (14 epicardial and 9 endocardial), indicate a strong correlation between the rate dependence of phase 1 amplitude and action potential duration.



Restitution of action potential duration in epicardium and endocardium. The results thus far presented indicate a close link between the spike and dome configuration of the epicardial response and the duration of the action potential under steady state stimulation conditions. Because the determinants of action potential duration are known to differ between steady state and nonsteady state stimulation conditions, we next evaluated the characteristics of restitution in the two types of tissue.

The two curves shown in Figure 6 depict the restitution of action potential duration in epicardial and endocardial prep-

Figure 6. Restitution of action potential duration (APD_{90}) in epicardium and endocardium. The action potential duration of premature responses elicited once after every 10th basic beat is plotted as a function of the diastolic interval (interval between the end of the basic action potential and the start of the premature response). The **shaded area** represents the action potential duration differences between the two types of tissue. The basic cycle length is 2,000 ms.



JACC Vol. 14, No. 4
October 1989:1053–66

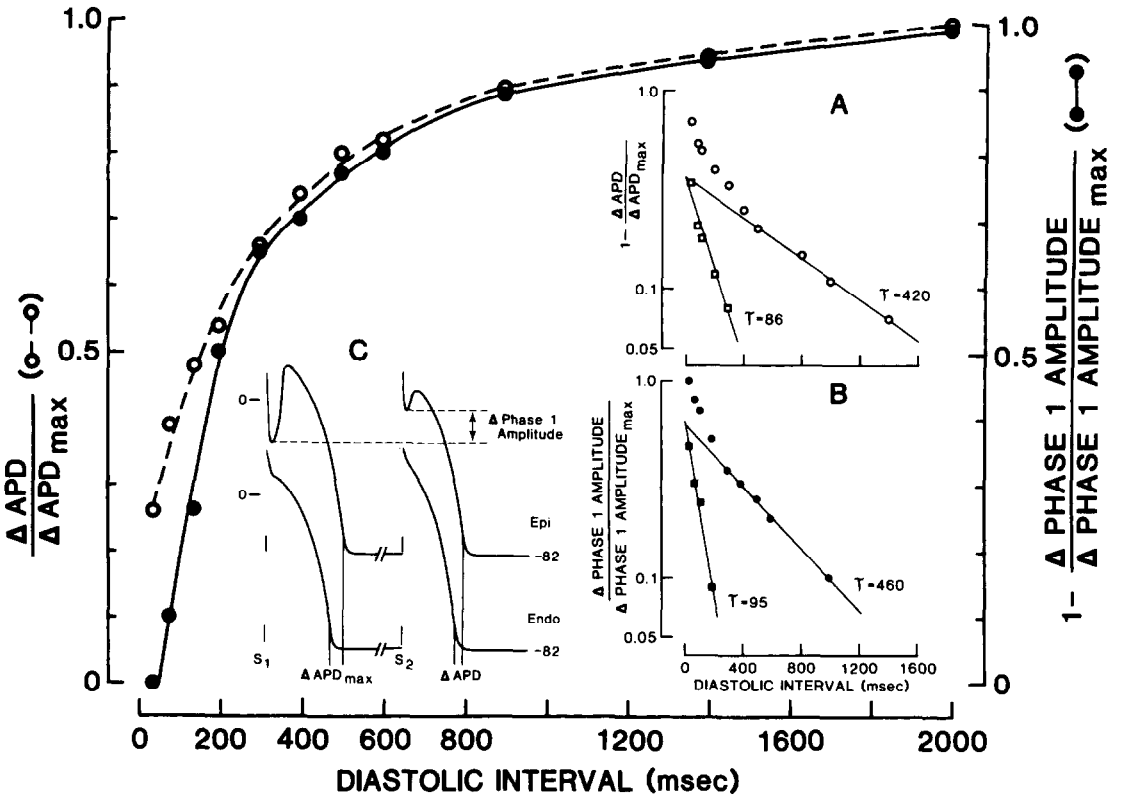


Figure 7. Comparison of the time dependence of the differences in action potential duration (Δ APD) between epicardium (epi) and endocardium (endo) with the time-dependent recovery of phase 1 amplitude in epicardium. Δ APD, the shaded area in Figure 6, is represented by **open circles** (values normalized to the maximal action potential duration difference between epicardium and endocardium). The **solid circles** represent the recovery of phase 1 amplitude in epicardium. Both are plotted as a function of the diastolic interval. The two curves are nearly superimposed. **Insets A and B** show the method used to derive τ (semilog plot). Each relation is well fitted by two exponential processes displaying brief and long time constants (τ). The respective τ values are similar for the two curves. **Inset C** shows simultaneous tracings from epicardium and endocardium denoting measurements of APD and phase 1 amplitude. The basic cycle length is 2,000 ms. max = maximal.

arations from the same heart. The action potential duration of premature beats elicited once after every 10th basic beat (basic cycle length = 2,000 ms) are plotted as a function of the diastolic interval. Once again, we observed a steeper relation for epicardium (top curve) than for endocardium. Another similarity to the results obtained with steady state stimulation is that in endocardium action potential duration reaches a maximal value early in diastole, whereas that of the epicardium continues to increase gradually throughout the diastolic period. In six of nine endocardial preparations, action potential duration achieved a steady state within 300 ms; in three preparations, it declined again in late diastole.

To examine to what extent these tissue-specific differences may be related to differences in the time dependence of the early phases of the action potential, we compared the action potential duration differences between epicardium and endocardium during restitution (shaded region in Fig. 6) with the recovery characteristics of phase 1 amplitude in epicardium. Figure 7 shows that the two relations are largely superimposed and that both can be fitted by two exponential components with brief and long time constants. Similar results were obtained in two other experiments in which a similar analysis was performed. Because the restitution of phase 1 amplitude has been shown to be a reasonable measure of the recovery of the transient outward current in epicardium (9), the parallelism of the two variables shown in Figure 7 provides further support for the hypothesis that the characteristics of restitution of action potential duration differ in the two types of tissue because of the presence of a prominent transient outward current in epicardium that is lacking in endocardium.

Effect of 4-aminopyridine on the restitution of action potential duration in epicardium and endocardium. As a further test of this hypothesis, we examined the effect of this drug on the characteristics of restitution of action potential duration. Figure 8 shows traces of the basic (S_1) and earliest premature (S_2) responses recorded from an epicardial preparation under control conditions and after the addition of 4-aminopyridine. In the control period, the pronounced spike and dome configuration displayed by the basic beat is virtually absent in the premature response because little reactivation

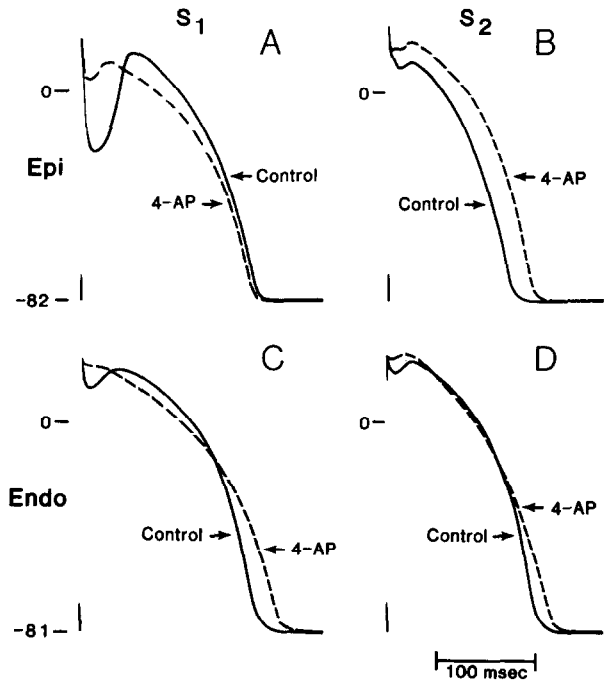


Figure 8. Comparison of the effects of 4-aminopyridine (4-AP, 1 m*M*) on the basic (S_1) and earliest premature (S_2) action potentials in epicardium (Epi) and endocardium (Endo). In epicardium, 4-aminopyridine abbreviates the basic action potential but increases the duration of the premature response. In endocardium, 4-aminopyridine prolongs the action potential duration of both the basic and premature beats. The basic cycle length is 2,000 ms.

of the outward current had occurred at the time the premature response was elicited. The action potential duration difference between the basic and premature action potentials was 54 ms. The addition of the outward current blocker greatly reduced the spike and dome of the basic response in epicardium, but had a much smaller effect on the early phases of the premature action potential (Fig. 8). These changes were attended by an abbreviation of the action potential duration of the basic beat, but a prolongation of that of the earliest premature beat; the duration difference between the basic and premature action potentials was thus reduced to 29 ms. In endocardium, 4-aminopyridine produced little change in the early phases of the action potential but acted to prolong the action potential duration of both the basic and premature beats.

Figure 9 graphically illustrates the effect of 4-aminopyridine on the complete action potential duration-restitution relations. Under control conditions (Fig. 9A), epicardium displays a more pronounced time dependence in early diastole and a progressive prolongation of action potential duration throughout the diastolic period, whereas endocardium achieves a steady state value within 500 ms. After 4-aminopyridine, the restitution curve for endocardium shifts upward and a biphasic configuration develops. In epicardium, the drug produced an upward shift in the early part of the restitution curve but a downward shift in the late part. Moreover, it eliminated the progressive increase of action potential duration in late diastole seen in control and produced a biphasic relation similar to that observed in endocardium. After 4-aminopyridine, the epicardial and endocardial restitution curves were nearly superimposed when normalized (not shown).

Table 2 presents a summary of the effects of 4-aminopyridine on epicardial and endocardial action potential variables of the basic and earliest premature beats. In epicardium, the drug's effects on the early phases of the action potential were much more pronounced for the basic beat than for the premature beat. It produced a nonsignificant abbreviation of the action potential duration of the basic beats due to the small n (Table 1), but a significant prolongation of the duration of the premature action potentials. All changes in action potential variables due to prematurity were significantly reduced by 4-aminopyridine. In endocardium, it produced a very small, but significant, change in the early phases of the basic action potentials but no change in those of the premature beats. Its effect of prolonging the duration of the action potential was more accentuated in the basic beats, as compared with the premature beats. As a result, the action potential duration difference between the basic and premature beats increased after 4-aminopyridine.

Using data collected from 11 epicardial and 9 endocardial preparations, we looked for a relation between the degree of phase 1 amplitude changes and action potential duration changes observed during restitution (Fig. 10). There was a significant correlation between the two variables, suggesting that tissue-specific differences, as well as interfiber variability (epicardium), in the degree of time dependence of action potential duration may, in large part, be attributable to differences in the relative contribution of the outward current. Inhibition of the latter with 4-aminopyridine caused a marked decrease in the magnitude and time dependence of phase 1 and a large decrease in the time dependence of action potential duration in epicardium (not shown).

Discussion

It is well known that a change in the pattern of stimulation usually modifies the duration of the action potential in cardiac tissues. This behavior is thought to be due to 1) incomplete recovery of ionic currents and a contribution from electrogenic pump currents between one action potential and the next; and 2) changes in activity of the ions in the intracellular and extracellular compartments (3,5,7,12). The first is primarily invoked to explain changes that occur with isolated premature beats or during the first few beats after an abrupt change of rate, whereas the second accounts for the

JACC Vol. 14, No. 4
October 1989:1053–66

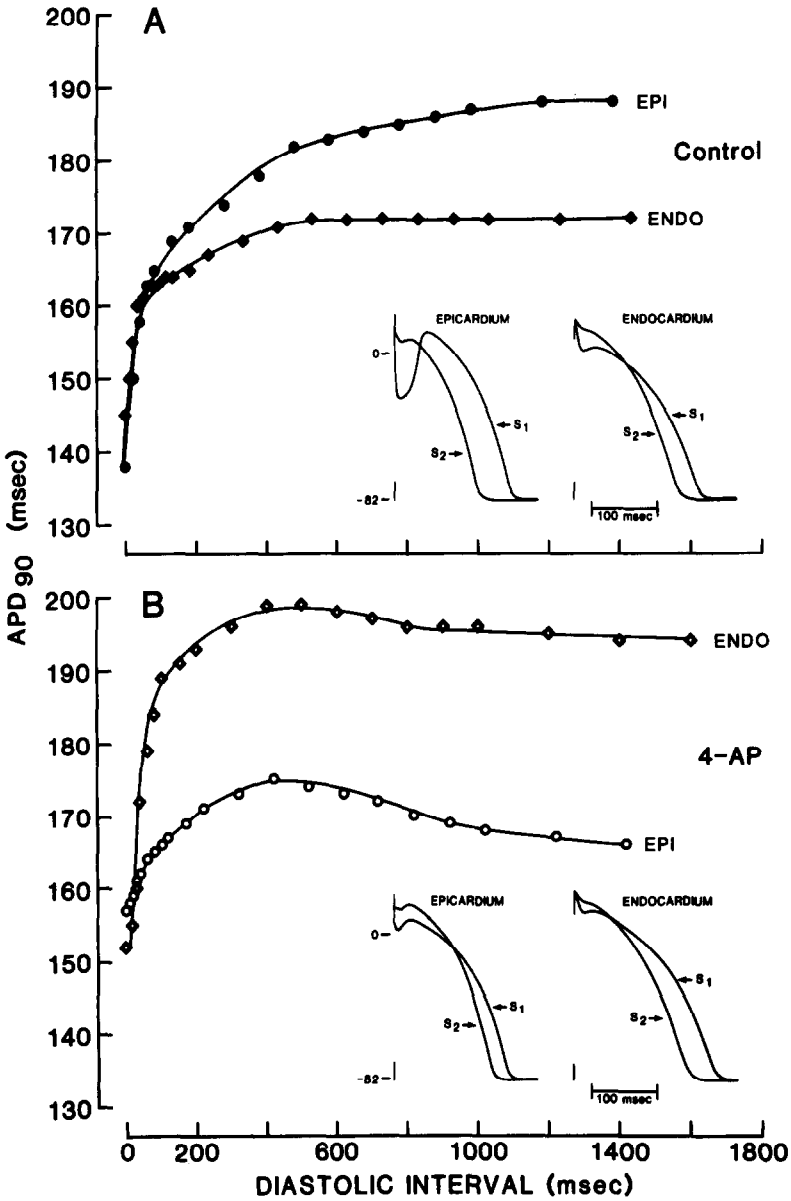


Figure 9. Effect of 4-aminopyridine (4-AP, 1 m*M*) on the restitution of action potential duration (APD_{90}) in epicardium (EPI) and endocardium (ENDO). **A,** Control study. Action potential duration of the premature beat is plotted as a function of the diastolic interval. **B,** Study recorded after addition of 4-aminopyridine. **Insets** show superimposed traces of the basic (S_1) and earliest premature (S_2) beats. In epicardium, 4-aminopyridine eliminates the time-dependent increase of action potential duration in late diastole and produces a downward shift of that part of the curve. In endocardium, it shifts the restitution curve upward; thus, the relative positions of the two curves on the ordinate axis are reversed after 4-aminopyridine. The basic cycle length is 2,000 ms.

slower and more gradual changes in action potential duration that develop after a change in rate.

The demonstration of a prominent time-dependent outward current in canine ventricular epicardium, but not endocardium (9), prompted us to examine the time and rate dependence of action potential duration and refractoriness in these two tissue types. The present study demonstrates that a significant heterogeneity exists in the response of these two tissues to changes in stimulation rate, and presents evidence to support the hypothesis that these differences are, in large part, secondary to differences in the relative contribution of the outward current to the active generator properties of the cells.

Characteristics of the transient outward current. The transient outward current has been described in both cardiac and noncardiac tissues (12–14) and has been assigned various names, including positive dynamic current, chloride current, initial outward current and early outward current. In the heart it has been reported in sheep (15–18), calf (19) and dog (20) Purkinje fibers, rabbit (21) and rat (22) ventricular myocardium, dog epicardium (9) and human atrial tissues (23). Recent studies have also described this current in isolated rat (22,24) and dog (25) ventricular myocytes, rabbit atrioventricular node cells (26), crista terminalis cells (29) and canine Purkinje cells (28). It appears to be largely absent in canine endocardium (9,20) and sheep and calf myocardium (29). The transient outward current is believed to be predominantly carried by K^+ ions (15,18,24,29) and shows voltage-dependent activation, inactivation and reactivation (15–17,19,22–28). A calcium-activated component has been reported (12,15,19).

Table 2. Effect of 1 m*M* 4-Aminopyridine (4-AP) on Action Potential Parameters of the Basic (S_1) and Earliest Premature Beats (S_2)

	S_1		S_2		S_1-S_2	
	Control	4-AP	Control	4-AP	Control	4-AP
Epicardium (n = 6)						
Rest potential (−mV)	80.3 ± 0.8	80.3 ± 0.5	80.3 ± 0.8	80.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Amplitude						
Phase 0 (mV)	89.1 ± 3.4	95.3 ± 2.8†	95.9 ± 2.7	97.1 ± 2.0*	−6.7 ± 1.5	−1.9 ± 1.3†
Phase 1 (mV)	61.9 ± 5.1	88.1 ± 6.8	86.3 ± 4.2	92.6 ± 3.5†	−24.4 ± 4.4	−5.9 ± 2.6†
Phase 2 (mV)	93.6 ± 2.7	90.9 ± 5.6	91.4 ± 2.6	94.6 ± 3.4†	2.1 ± 2.6	−3.7 ± 3.4†
Phase 1 magnitude (mV)	27.3 ± 6.5	8.6 ± 4.7†	9.6 ± 3.8	4.6 ± 2.8†	17.7 ± 5.2	4.0 ± 2.1†
APD_{50} (ms)	150.7 ± 11.0	143.4 ± 10.6	108.9 ± 12.2	117.0 ± 8.8†	41.9 ± 4.8	26.4 ± 4.8†
APD_{90} (ms)	180.4 ± 11.8	173.9 ± 14.4	136.9 ± 14.6	147.1 ± 13.0†	43.6 ± 5.5	26.9 ± 4.2†
Endocardium (n = 4)						
Rest potential (−mV)	81.8 ± 2.2	81.8 ± 2.2	81.8 ± 2.2	81.8 ± 2.2	0.0 ± 0.0	0.0 ± 0.0
Amplitude						
Phase 0 (mV)	105.8 ± 3.0	107.0 ± 2.7†	107.0 ± 2.7	107.3 ± 2.5	−1.3 ± 0.5	−0.3 ± 0.5*
Phase 2 (mV)	97.5 ± 4.1	99.5 ± 2.9*	100.0 ± 3.3	100.5 ± 3.7	−2.5 ± 1.0	−2.0 ± 1.4
APD_{50} (ms)	138.8 ± 7.5	155.3 ± 10.0*	111.8 ± 11.8	122.5 ± 16.9*	27.0 ± 5.0	32.8 ± 7.8*
APD_{90} (ms)	168.5 ± 10.8	184.8 ± 14.7*	140.3 ± 14.9	149.3 ± 18.7*	28.3 ± 5.0	35.5 ± 6.8*

Symbols and abbreviations as in Table 1.

With use of the restitution of the amplitude of phase 1 of the action potential as a marker for the reactivation of outward current, two exponential processes have been delineated in canine ventricular epicardium: 1) a slow component that recovers with a time constant (τ) of 350 to 570 ms and is largely abolished by the outward current blocker, 4-aminopyridine (1 to 5 m*M*); and 2) a fast component with a τ of 41 to 85 ms that is diminished by 4-aminopyridine, but is also inhibited by ryanodine and by strontium (Sr^{+2}) replacement of calcium (Ca^{+2}) interventions known to inhibit the Ca^{+2}-activated component of the transient outward current (9,19). Tseng et al. (25) recently demonstrated two components of this outward current, with widely different kinetics and pharmacologic sensitivities in isolated canine ventricular myocytes, that displayed a spike and dome (approximately half of the cells isolated from transmural segments). The presence of two such components with brief and long reactivation and inactivation time constants has now been described in a large variety of cardiac tissues and cells (15,16,19,26–28).

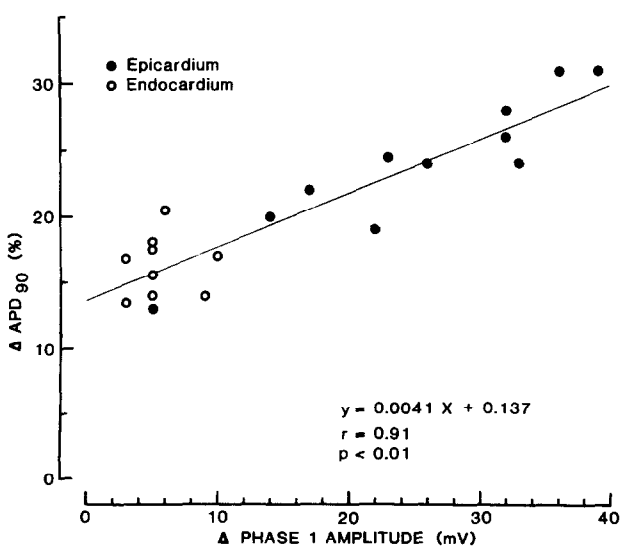


Figure 10. Relation between the time dependence of action potential duration (APD) and the time dependence of phase 1 amplitude in epicardium and endocardium. The difference between the action potential duration of the basic and earliest premature beats (Δ APD_{90}, expressed as percent abbreviation of the action potential duration of the basic beat) is plotted as a function of the change in phase 1 amplitude of the same two responses. The data are from 20 different preparations (11 epicardial and 9 endocardial). The basic cycle length is 2,000 ms.

Rate and time dependence of action potential duration. Our data indicate that the rate dependence of action potential duration is clearly more pronounced in epicardium than in endocardium, and that the action potential duration rate relations of the two tissues cross over at a basic cycle length of approximately 1,000 ms (Fig. 2A and 4A) resulting in an epicardial action potential duration that is briefer than that of endocardium at rapid stimulation rates, but is longer at slow rates. As might be expected, changes in the effective refractory period paralleled those of action potential duration in both tissues.

Another distinguishing feature was that the action potential duration-rate relation of endocardium approached a steady state at basic cycle lengths >1,000 ms, whereas that of epicardium continued to rise with progressive deceleration. This behavior of canine ventricular endocardium is consistent with that reported by Miller et al. (8) but not with the results of others (6,30) who reported a progressive

JACC Vol. 14, No. 4
October 1989:1053–66

prolongation of endocardial action potential duration with deceleration. In our study, as in that of Miller et al. (8), great care was taken to avoid or exclude data from transitional cells whose action potential characteristics and rate-dependent behavior are more akin to those of Purkinje fibers. Indeed, our data (unpublished observations) collected from transitional cells at the base of the papillary muscles were similar to those previously reported (6,30).

Epicardium and endocardium also differ with respect to the characteristics of the recovery of action potential duration. Whereas the restitution curves derived from endocardium reach a steady state early in diastole, those of epicardium exhibit a progressive prolongation of action potential duration throughout the diastolic period (Fig. 6 and 9A). The endocardial data are consistent with previous studies (1–3,8,31,32). Although, to our knowledge, there are no reports of the characteristics of restitution in epicardium, our findings are congruous with those recently reported by Robinson et al. (32), who observed large variability in the magnitude of the late component of the restitution curves. Our results suggest that the cells that displayed a time-dependent increase of action potential duration in late diastole are those representative of epicardial origin, whereas those that lacked this behavior are representative of endocardium.

Does the transient outward current contribute to the time and rate dependence of action potential duration? Several lines of evidence point to an important influence of outward current on the action potential duration of canine ventricular epicardium: 1) There is a strong correlation between the rate dependence of action potential duration and the rate dependence of phase 1 amplitude (the action potential variable most representative of outward current availability) among the different epicardial preparations studied (Fig. 5). 2) A direct relation also exists between the recovery of action potential duration and the recovery of phase 1 amplitude among a population of epicardial cells showing wide variability in the prominence of the spike and dome configuration of the action potential (Fig. 10). 3) 4-Aminopyridine blockade of outward current produces corresponding changes in the time dependence of phase 1 amplitude and the time dependence of action potential duration (Fig. 7 to 9). 4) 4-Aminopyridine alters the action potential and the action potential duration-rate relation of epicardium in such a way that both resemble more closely those of endocardium (Fig. 3 and 4). Finally, 4-aminopyridine does not alter significantly the shape of the action potential duration-rate relation nor the shape of the restitution curve of endocardium, a tissue in which outward current is thought to be largely lacking (Fig. 4B).

Does the prominent presence of a transient outward current in epicardium, but not endocardium, account for the differences in the time and rate dependence of action potential duration in the two tissues? That the transient outward current may, in large part, be responsible for the difference in behavior between epicardium and endocardium is suggested by the following observations: 1) When exposed to 4-aminopyridine, the action potential duration-rate relations and restitution curves of the two tissues become nearly superimposed when normalized (Fig. 4 and 9). 2) Time-dependent changes of action potential duration differences between the two tissues can be fitted by a biexponential whose two components show time constants very similar to those of the two components that describe the time dependence of phase 1 amplitude in epicardium (Fig. 6 and 7). Endocardium fits well into the lowest extreme of the relation between the time or rate dependence of action potential duration and phase 1 amplitude in epicardium (Fig. 5 and 10).

Does the transient outward current abbreviate the action potential in epicardium? Although the presence of an additional outward current might be expected to abbreviate the epicardial action potential, our data suggest otherwise. The action potential duration of epicardium is generally briefer than that of endocardium only at relatively rapid stimulation rates. Under these conditions, the contribution of the transient outward current to the active generator properties of the epicardial cell is relatively small because of the slow recovery of this current from inactivation. At progressively slower rates, as the availability of the outward current increases, the epicardial action potential becomes progressively longer than that of endocardium (Fig. 2 and 4). Moreover, during restitution, the action potential duration of epicardium is generally longer than that of endocardium throughout diastole when the two preparations studied are from the same heart (Fig. 6 and 9). Finally, inhibition of the transient outward current with 4-aminopyridine, at concentrations (0.5 to 1 m*M*) known to produce a relatively small inhibition of the inward rectifier (I_{K1}), results in little change in the action potential duration of epicardium at fast rates (when the availability of the transient outward current is small) but a significant abbreviation of the epicardial action potential at slower stimulation rates (when the availability of the current is much greater). In contrast, 4-aminopyridine always prolonged the action potential duration of endocardium, as well as that of the earliest premature beats in epicardium, consistent with a predominant effect of the drug on the inward rectifier (background potassium current) (33) when the transient outward current is largely absent. Thus, our data clearly indicate that the transient outward current exerts a prolonging influence on the epicardial action potential.

Possible mechanisms for the transient outward current-action potential duration interplay in epicardium. Figure 1 provides some insight into the mechanism by which the transient outward current acts to prolong action potential duration in epicardium. At the slower stimulation rate, a more intense transient outward current gives rise to a steeper phase 1 in the epicardial action potential. Phase 2 is thus initiated at a more negative potential when the preparation is stimulated at slower rates, and the peak plateau (peak of phase 2) is

achieved later because more time is required to effect the greater voltage swing.

Several factors may contribute to this phenomenon in epicardium. In view of the rapid inactivation of the transient outward current, as demonstrated in canine Purkinje and ventricular myocytes (25,28) and the foregoing discussion, it seems unlikely that the transient outward current exerts much of an influence on the repolarization phase (phase 3) of the epicardial action potential. The main influence of the transient outward current on action potential duration appears to be secondary to the effect of this current on the early phases of the epicardial action potential. At progressively slower rates of stimulation, as the availability of this outward current increases, phase 1 becomes more accentuated, leading to progressively more negative potentials at which phase 2 is initiated. At the more negative potentials, the second upstroke is initially slow to develop (Fig. 3) and peak plateau voltage achieved is usually more positive. The slow development of the initial part of the second upstroke might be related to the low availability or slow kinetics of the slow inward current (calcium current) at these potentials (as low as −30 mV). The reason for the more positive plateau is not clear but may be related to a slower activation of the delayed rectifier (I_x,I_K) under these conditions or a slower inactivation of the slow inward current (34). Alternatively, this phenomenon may be related to a greater level of slow inward current at the slow rates (35–36). The more positive plateau at the slower stimulation rates and with late coupled beats, however, is unexpected on the basis of rate dependence and restitution characteristics of the slow inward current (37–38).

Physiologic and Clinical Implications

Our data provide further support for the existence of a marked heterogeneity of active membrane properties and electrophysiologic characteristics in ventricular myocardium. The available data suggest that this heterogeneity may have far-reaching implications with respect to our understanding of some aspects of cardiac electrophysiology, electrocardiography, pathophysiology and pharmacology.

Implications in electrophysiology and electrocardiography. The differences in the responsiveness of epicardium and endocardium to changes in rate may add to our understanding of the basis for rate-dependent changes in the T wave of the electrocardiogram (ECG), as well as our understanding of rate-dependent changes in the manifestation of some forms of cardiac arrhythmia, intramural reentry in particular. Our findings indicate that a dispersion of repolarization, as well as of refractoriness, exists across the ventricular wall of the canine heart and that the magnitude and direction of the dispersion are rate dependent.

In recent studies we have also uncovered a differential sensitivity of these two tissues to changes in $[K^+]_o$ (39). Although K^+-dependent changes in action potential amplitude (phase 0) and rest membrane potential are similar in the two tissues, changes in action potential duration are more pronounced in epicardium than in endocardium after an increase of $[K^+]_o$ from 2 to 8 m*M*. This difference is greatly reduced or eliminated in the presence of the outward current blocker, 4-aminopyridine. Of relevance to the present study is the observation that the crossover of the action potential duration-rate relations of the two tissues shifts to progressively longer basic cycle lengths, as $[K^+]_o$ is increased, and to shorter cycle lengths when $[K^+]_o$ is decreased (Fig. 2). The results suggest that the presence of a prominent transient outward current in epicardium, but not in endocardium, contributes to a greater sensitivity of epicardium to changes in $[K^+]_o$ and that differences in the response of the two tissues to $[K^+]_o$ may, in part, account for the classical ECG patterns that attend hypokalemia and hyperkalemia.

In epicardium, but not in endocardium, premature stimulation evokes action potentials whose amplitudes are greatly augmented (Fig. 9). This behavior may provide an explanation for supernormal conduction in ventricular epicardium. The possibility of supernormal conduction or excitability in ventricular muscle has long been a matter of debate. In vivo studies (40–41) employing extracellular stimulating electrodes (in many cases applied to the ventricular epicardium) have demonstrated a supernormal period, whereas studies of ventricular muscle tissues (endocardium) in vitro have failed to uncover a period of supernormal excitability (42). Supernormal conduction has not been demonstrated previously in isolated ventricular muscle tissues. In view of our recent findings, we considered the hypothesis that, under conditions of impaired conduction or anisotropy, supernormal conduction may occur in epicardium because of the much greater local circuit current provided by the augmented response in early versus late diastole. Using a sucrose gap to create a zone of depressed conductivity, we recently succeeded in demonstrating a supernormal phase of conduction in isolated canine epicardium but not endocardium (43). These observations, coupled with those already discussed, suggest that changes in stimulation protocol may lead to very different changes in conduction as well as refractoriness in epicardium versus endocardium under pathophysiologic conditions.

Implications in myocardial ischemia. Previous studies have demonstrated that canine ventricular epicardium is more sensitive than endocardium to electrical depression during ischemia (44–45). In vivo studies of acute myocardial ischemia have also shown that conduction in the endocardium remains relatively preserved at a time when conduction in epicardium has become increasingly delayed and fractionated (46–50). In a parallel study (43,51) we have shown that the presence of an additional outward current in epicardium is, in large part, responsible for the differential sensitivity of these two tissues to ischemic conditions. An important

JACC Vol. 14, No. 4
October 1989:1053–66

finding is that the ischemia-induced electrical depression (loss of the action potential plateau) in epicardium can be reversed by acceleration of the stimulation rate. This paradoxical effect is consistent with the fact that the transient outward current is slow to reactivate and that its availability is, therefore, greatly diminished at rapid rates. Under ischemic conditions, acceleration may prolong refractoriness in epicardium and could potentially improve conduction. These rate-dependent changes are not observed in endocardium.

Pharmacologic implications. Electrophysiologic differences between epicardium and endocardium can also contribute to differences in the responsiveness of these two tissues to a variety of pharmacologic agents. On the basis of the previous discussion of a critical interplay between the transient outward current and the inward calcium current in epicardium, it seems reasonable to suggest that any agent that exerts an effect on either current may elicit different responses in epicardium and endocardium. The differential response to the outward current blocker, 4-aminopyridine, is one example (Fig. 3, 4, 8 and 9). The response to acetylcholine is another. Through its actions to block the calcium inward current, acetylcholine produces major changes in the epicardial action potential at concentrations that have little or no effect on endocardium (43). Concentrations of acetylcholine as high as 10^{-4} *M* produce little change in the endocardial action potential. In epicardium, however, acetylcholine produces prominent dose-dependent and rate-dependent changes in the configuration of the action potential. Physiologically relevant concentrations act to prolong the epicardial action potential secondary to an accentuation of the spike and dome. These effects of acetylcholine are reversed by 4-aminopyridine and are not observed in epicardial preparations pretreated with 4-aminopyridine. Isoproterenol, among its many actions, augments the calcium inward current. Its effects on the early phases of the epicardial action potential are opposite to those of acetylcholine (43). In a concentration of 6×10^{-6} *M*, isoproterenol abbreviates the action potential and refractory period to a greater extent in epicardium than in endocardium.

Quinidine is another agent that produces different electrophysiologic changes in epicardium and endocardium (52). Quinidine's antiarrhythmic activity is generally ascribed to its effects to block the fast sodium and late potassium currents. Imaizumi and Giles (53) recently reported that quinidine also blocks the transient outward current in rabbit cardiac cells. Quinidine's effect of prolonging the action potential and refractory period in the canine ventricle is more pronounced in endocardium than in epicardium. In epicardium, but not in endocardium, the changes in action potential duration are accompanied by quinidine-induced changes in the early phases of the action potential. These findings suggest that quinidine may also inhibit the transient outward current in canine epicardium and that this action may, in part, explain the differences in the responsiveness of epicardium and endocardium to the drug. Thus, the intrinsic disparity in the rate dependence of repolarization and refractoriness between epicardium and endocardium can be importantly influenced, and in some cases greatly exaggerated, by differential responsiveness of the two tissues to a variety of drugs and neurotransmitters.

Prominent J wave in electrocardiogram (ECG): role of hypothermia and hypercalcemia. Finally, the presence of a prominent spike and dome in ventricular epicardium, but not endocardium, might be expected to produce a voltage gradient during ventricular activation that should manifest in the ECG as a low amplitude, late delta wave or what is commonly referred to as a J wave (Osborne wave). Although the J wave is not commonly encountered in the standard ECG, signal averaged recordings often show a deflection at the end of the QRS complex thought to be representative of the J wave. The appearance of a prominent J wave in the standard ECG is a well known feature of hypothermia (54). A possible explanation for this phenomenon is that the spike and dome configuration of the epicardial response becomes more prominent at low temperatures, whereas the early phases of the endocardial response are unaffected by the change in temperature. In support of this hypothesis is the observation of West et al. (55) nearly 30 years ago of a marked accentuation of the spike and dome configuration of action potentials recorded from the epicardial surface of canine ventricles in situ under hypothermic conditions. As a further test of the hypothesis, we examined the effects of temperature on the action potential characteristics of isolated canine ventricular epicardial and endocardial preparations (43). We found a marked accentuation of the spike and dome configuration of the epicardial action potential, with little change in the early phases of the endocardial response during cooling.

Hypercalcemia is another condition recently reported to give rise to a J wave in the standard ECG (56). No explanation for this phenomenon has been advanced. In preliminary experiments employing isolated canine ventricular epicardial and endocardial preparations, we observed an accentuation of the spike and dome configuration of the epicardial action potential and a positive shift in the voltage of the plateau of the endocardial action potential in response to an elevation of the calcium concentration. These observations suggest that differences in the response of epicardium and endocardium to temperature and $(Ca^{++})_o$ may underly the appearance of a J wave in the ECG under hypothermic and hypercalcemic conditions. If this hypothesis is correct, our present data suggest that the manifestation of a J wave or perhaps an elevated J point should show a heart rate dependence.

We acknowledge the expert technical assistance of Judith Hefferon and Robert Goodrow.

References

1. Greenspan K, Edmands RE, Fisch C. Effects of cycle length alteration on canine cardiac action potentials. Am J Physiol 1967;212:1416–20.
2. Bass BG. Restitution of the action potential in cat papillary muscle. Am J Physiol 1975;228:1717–24.
3. Boyett MR, Fedida D. The effect of heart rate on the membrane currents of isolated sheep Purkinje fibres. J Physiol 1988;399:467–91.
4. Boyett MR, Jewell BR. A study of the factors responsible for rate dependent shortening of the action potential in mammalian ventricular muscle. J Physiol 1978;285:359–80.
5. Boyett MR, Jewell BR. Causes of rate-dependent changes of electrical activity: progress in biophysic and molecular biology. Prog Biophys Mol Biol 1980;36:1–52.
6. Elharrar V, Surawicz B. Cycle length effect on restitution of action potential duration on dog cardiac fibers. Am J Physiol 1983;244:782–92.
7. Carmeliet E. Repolarization and frequency in cardiac cells. J Physiol (Paris) 1977;73:903–23.
8. Miller JP, Wallace AG, Feezor MD. A quantitative comparison of the relation between the shape of the action potential and the pattern of stimulation in canine ventricular muscle and Purkinje fibers. J Mol Cell Cardiol 1971;2:3–19.
9. Litovsky SH, Antzelevitch C. Transient outward current prominent in canine ventricular epicardium but not endocardium. Circ Res 1988; 62:116–26.
10. Yanagisawa T, Satoh K, Taira N. Excitation of autonomic nerves by 4-aminopyridine in the isolated, blood perfused sinoatrial node preparation of the dog. Eur J Pharmacol 1978;49:189–92.
11. Meves H, Pichon Y. The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. J Physiol 1977;268:511–32.
12. Cohen IS, Datyner NB, Gintant GA, Kline RP. Time dependent outward currents in the heart. In: Fozzard HA, Jennings RB, Haber E, Katz AM, Morgan HE, eds. The Heart and Cardiovascular System. New York: Raven Press, 1986:637–70.
13. Mackenzie E, Standen NB. The effect of stimulation rate on calcium-dependent action potentials recorded from chick embryo blast cell aggregates. J Physiol 1982;324:1–10.
14. Belluzzi O, Sacchi O, Wanke E. A fast transient outward current in the rat sympathetic neurone studied under voltage-clamp conditions. J Physiol 1985;358:91–108.
15. Coraboeuf E, Carmeliet E. Existence of two transient outward currents in sheep cardiac Purkinje fibers. Pflugers Arch 1982;392:352–9.
16. Fozzard HA, Hiraoka M. The positive dynamic current and its inactivation properties in cardiac Purkinje fibers. J Physiol 1973;234:569–86.
17. Kenyon JL, Gibbons WR. 4-Aminopyridine and the early outward current of sheep Purkinje fibers. J Gen Physiol 1979;73:139–57.
18. Boyett MR. Effect of rate dependent changes in the transient outward current on the action potential in sheep Purkinje fibers. J Physiol 1981;319:23–41.
19. Siegelbaum SA, Tsien RW. Calcium activated transient outward current in calf cardiac Purkinje fibres. J Physiol 1980;299:485–506.
20. Gilmour RF, Salata JJ, Davis JR. Effect of 4-aminopyridine on rate related depression of cardiac action potentials. Am J Physiol 1986;251:H297–306.
21. Kukushkin NI, Gainullin RZ, Sosunov EA. Transient outward current and rate dependence of action potential duration in rabbit cardiac ventricular muscle. Pflugers Arch 1983;399:87–92.
22. Watanabe T, Dellbridge LM, Bustamante JO, McDonald TF. Heterogeneity of the action potential in isolated rat ventricular myocytes and tissue. Circ Res 1983;52:280–90.
23. Escande D, Loisance D, Planche C, Coraboeuf E. Age-related changes of action potential plateau shape in isolated human atrial fibers. Am J Physiol 1985;249:H843–50.
24. Josephson JR, Sanchez Chapula J, Brown AM. Early outward current in rat single ventricular cells. Circ Res 1984;54:157–62.
25. Tseng G-N, Hoffman BF. Two components of transient outward current in canine ventricular myocytes. Circ Res 1989;64:633–47.
26. Nakayama T, Irisawa H. Transient outward current carried by potassium and sodium in quiescent atrioventricular node cells of rabbits. Circ Res 1985;57:65–73.
27. Giles WR, Van Ginneken ACG. A transient outward current in isolated cells from the crista terminalis of rabbit heart. J Physiol 1985;368:243–64.
28. Nakayama T, Fozzard HA. Adrenergic modulation of the transient outward current in isolated canine Purkinje cells. Circ Res 1988; 62:162–72.
29. Beeler GW, Reuter H. Reconstruction of the action potential of ventricular myocardial fibers. J Physiol 1977;268:177–210.
30. Moore EN, Preston JB, Moe GK. Durations of transmembrane action potentials and functional refractory periods of canine false tendon and ventricular myocardium: comparisons in single fibers. Circ Res 1965; 17:259–73.
31. Iinuma H, Kato K. Mechanism of augmented premature responses in canine ventricular muscle. Circ Res 1979;44:624–9.
32. Robinson RB, Boyden PA, Hoffman BF, Hewett KW. Electrical restitution process in dispersed canine cardiac Purkinje and ventricular cells. Am J Physiol 1987;253:1018–25.
33. Van Bogaert PP, Snyders DJ. Effects of 4-aminopyridine on inward rectifying and pacemaker currents of cardiac Purkinje fibers. Pflugers Arch 1982;394:230–8.
34. Kass RS, Tsien RW. Control of action potential duration by calcium ions in cardiac Purkinje fibers. J Gen Physiol 1976;67:599–617.
35. Simurda J, Simurdova M, Braveny P, Sumbera J. Slow inward current and action potentials of papillary muscles under non-steady state conditions. Pflugers Arch 1976;362:209–18.
36. Gibbons WR, Fozzard HA. Slow inward current and contraction of sheep cardiac Purkinje fibers. J Gen Physiol 1975;65:367–84.
37. Mitra R, Morad M. Two types of calcium channels in guinea pig ventricular myocytes. Proc Natl Acad Sci USA 1986;83:5340–4.
38. Tseng GN, Hoffman BF. A novel mechanism controlling Ca current repriming in mammalian ventricular cells (abstr). Biophys J 1987;51:410a.
39. Litovsky SH, Antzelevitch C. Differences in electrophysiologic responses of canine epicardium and endocardium to changes in $[K^+]_o$ may explain classical ST-T changes (abstr). FASEB J 1988;2:930a.
40. Burchell HB. Analogy of electronic pacemaker and ventricular parasystole with observation of refractory period, supernormal phase and synchronization. Circulation 1963;27:878–89.
41. Hernandez Pieretti O, Morales Rocha J, Barcelo JE. Supernormal phase of conduction in human heart demonstrated by subthreshold pacemaker. Br Heart J 1969;31:553–8.
42. Spear JF, Moore EN. Supernormal excitability and conduction in the His-Purkinje system of the dog. Circ Res 1974;35:782–92.
43. Antzelevitch C, Litovsky SH, Lukas A. Ventricular epicardium vs. endocardium: electrophysiology and pharmacology. In: Zipes D, Jalife J, eds. Cardiac Electrophysiology, From Cell to Bedside. New York: WB Saunders, 1989, in press.
44. Gilmour RF Jr, Zipes DP. Different electrophysiological responses of canine endocardium and epicardium to combined hyperkalemia, hypoxia, and acidosis. Circ Res 1980;46:814–25.
45. Kimura S, Basset AL, Kohya T, Kozlovskis PL, Myerburg RJ. Simultaneous recording of action potentials from endocardium and epicardium during ischemia in the isolated cat ventricle: relation of temporal electrophysiologic heterogeneities to arrhythmias. Circulation 1986;74:401–9.
46. Boineau JP, Cox JL. Slow ventricular activation in acute myocardial infarction: a source of re-entrant premature ventricular contractions. Circulation 1973;48:702–13.

47. Scherlag BJ, El-Sherif N, Hope R, Lazzara R. Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction. Circ Res 1974;35:372–83.

48. Williams DO, Scherlag BJ, Hope R, El-Sherif N, Lazzara R. The pathophysiology of malignant ventricular arrhythmias during acute myocardial infarction. Circulation 1974;50:1163–72.

49. Elharrar V, Gaum WE, Zipes DP. Effects of drugs on conduction delay and the incidence of ventricular arrhythmias induced by acute coronary occlusion in dogs. Am J Cardiol 1977;39:544–9.

50. Ruffy R, Lovelace ED, Knoebel SB, Mueller TM, Zipes DP. Relationship between changes in left ventricular bipolar electrograms and regional myocardial blood flow during acute coronary occlusion in the dog. Circ Res 1979;45:764–70.

51. Lukas A, Antzelevitch C. Does the transient outward current contribute to selective electrical depression of canine epicardium during ischemia (abstr)? FASEB J 1988;2:930a.

52. Antzelevitch C, Davidenko J, Sicouri S, et al. Early afterdepolarizations and triggered activity. J Electrophysiol 1989; in press.

53. Imaizumi Y, Giles WR. Quinidine-induced inhibition of the transient outward current in cardiac muscle. Am J Physiol 1987;253:H704–8.

54. Clements SD, Hurst JW. Diagnostic value of electrocardiographic abnormalities observed in subjects accidentally exposed to cold. Am J Cardiol 1972;29:729–34.

55. West T, Frederickson E, Amory D. Single fiber recording of the ventricular response to induced hypothermia in the anesthetized dog; correlation with multicellular parameters. Circ Res 1959;7:880–8.

56. Sridharan MR, Horan LG. Electrocardiographic J wave of hypercalcemia. Am J Cardiol 1984;54:672–3.