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SLOW RECOVERY FROM INACTIVATION OF INWARD CURRENTS IN MAMMALIAN MYOCARDIAL FIBRES

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SUMMARY

1. Reactivation kinetics of the rapid and slow inward currents in ventricular fibres have been assessed by studying the maximum rate of rise ($(dV/dt)max$) of the action potential upstroke and the duration of the plateau in progressively earlier premature responses. Reactivation of the slow inward current was also studied by voltage clamp technique in sheep and pig ventricular trabeculae.

2. The time constant of recovery of $(dV/dt)max$ was voltage dependent and increased from less than 20 msec when the resting membrane potential was more negative than -80 mV to more than 100 msec when the resting membrane potential was between -65 and -60 mV. Similar results were obtained in Purkinje fibres. These results suggest that the time constant for reactivation is slower than the time constant for inactivation of the rapid inward current system by at least one order of magnitude.

3. The time constant of recovery of plateau duration was also voltage dependent and increased from 30 to 70 msec as the membrane potential was changed from -85 to -60 mV.

4. The reactivation time constant of the slow inward current determined by voltage clamp experiments were similar to the results obtained by analysis of plateau duration. At potentials less negative than -60 mV the time constant of reactivation became progressively longer. Unlike reactivation time constants of $(dV/dt)max$, the time constants of reactivation of the slow inward current were similar to the time constants of inactivation.

5. Our results indicate that (*a*) in premature action potentials, time as well as voltage are important determinants of $(dV/dt)max$ in myocardial and Purkinje fibres, (*b*) the kinetics of reactivation of the rapid inward current in cardiac fibres are different from those in nerve and (*c*) plateau

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duration of premature action potentials in ventricular myocardial fibres is largely determined by the kinetics of reactivation of the slow calcium inward current.

INTRODUCTION

An understanding of factors which determine the rate of rise and the duration of the action potential is important if we are to understand the factors which determine conduction velocity and refractoriness in the intact heart. In ventricular myocardial fibres, the rapid upstroke and the plateau of the action potential are dependent on two inward current systems: (1) a rapid current carried by sodium ions which is primarily responsible for the rapid upstroke and (2) a slow current attributed primarily to calcium ions, which contributes to the terminal portion of the upstroke and to the plateau. Both current systems are voltage and time dependent (for review see Reuter, 1973).

In cardiac muscle, the sodium inward current and rapid upstroke of the cardiac action potential depend upon the transmembrane voltage at the onset of depolarization (Weidmann, 1955*a*). This dependence is analogous to the situation in nerve (Hodgkin & Huxley, 1952). It is thought that the recovery from inactivation of the sodium inward current is very rapid in cardiac muscle as in nerve and that the current regains its steady-state value following a preceding depolarization within a few msec. Thus, in premature responses as in the steady-state, the rapid upstroke of the cardiac action potential should depend primarily on the membrane potential at the onset of depolarization. However, there are several observations suggesting that in cardiac muscle the recovery of the rapid inward sodium system from inactivation may be rather slow when the membrane potential is less negative than -80 mV and that time may be an important factor in the recovery process.

Weidmann (1955*a*), utilizing (dV/dt)max as an index of sodium inward current, showed in spontaneously depolarizing Purkinje fibres, that (dV/dt)max of the action potential was slower when the responses originated from incompletely repolarized fibres than when the responses originated later in diastole from a similar membrane potential level. More recently, Strauss & Bigger (1972), illustrated that in atrial fibres of the crista terminalis and those fibres surrounding the SA node, (dV/dt)max of the action potential was slower in responses originating from incompletely repolarized fibres than in those originating from a similar membrane potential level when the fibres were depolarized with potassium chloride. Haas, Kern, Einwächter & Tarr (1971), showed in frog atria using voltage clamp techniques, that the recovery from inactivation of the rapid inward current was markedly prolonged as compared to inactivation of the current itself.

Inactivation of the slow, calcium dependent inward current is voltage dependent (Beeler & Reuter, 1970*b*; Reuter, 1973), and time constants of inactivation of up to several hundred msec have been reported when the membrane potential was in the positive potential range. The reactivation kinetics of the calcium current have not been described. However, studies of premature responses in pig ventricular fibres have shown that the duration of the premature ventricular myocardial action potential does not begin to shorten until the preceding diastolic interval is 100 msec or less (Gettes, Morehouse & Surawicz, 1972). This result suggests that the ionic currents responsible for the plateau may require 100 msec to recover from the activation–inactivation cycle.

In this study we have attempted to define the reactivation kinetics of the rapid and slow inward current systems by studying (dV/dt)max of the rapid upstroke and plateau duration of the action potential and by utilizing voltage clamp techniques to study the slow inward current. Our results indicate that reactivation of the sodium system is a much slower process in cardiac muscle than in squid axon and that the reactivation kinetics of the rapid and slow inward current systems are different. Our results also suggest that the kinetics of the slow inward calcium current can account for the plateau duration of the ventricular myocardial action potential in premature responses. Two short notes on this work have been communicated previously (Gettes & Reuter, 1972*a*, *b*).

METHODS

Right ventricular papillary muscles from guinea-pig hearts, thin (0·8 mm or less) ventricular trabeculae from sheep, calf and pig hearts, and free running Purkinje fibres from sheep and calf hearts were used in the study. The ventricular muscles were mounted in a three compartment 'sucrose-gap' chamber (Reuter & Scholz, 1968). The Purkinje fibres were mounted in a single compartment chamber. The sucrose-gap chamber was used in order to pass current through the preparation and to depolarize or hyperpolarize homogeneously the short (about 1 mm) proximal muscle end.

The proximal and distal compartments of the muscle chamber were perfused with normal Tyrode solution of the following composition (mM): NaCl 137; KCl 5·4; $CaCl_2$ 1·8; $MgCl_2$ 1·05; $NaHCO_3$ 11·9; NaH_2PO_4 0·42; glucose 5; gassed with 95 % O_2, 5 % CO_2. The middle compartment of the muscle chamber was perfused with isotonic sucrose solution. The Purkinje fibre chamber was perfused with Tyrode solution. All experiments were performed at 37° C. In addition, some were also performed at 27° C.

The preparation was stimulated at a basic rate of 0·25–0·5/sec. The rate was chosen so that (dV/dt)max of the 'conditioning' action potentials were at the steady-state. The transmembrane potential was measured in the proximal muscle end or in the Purkinje fibre relative to virtual ground with two conventional glass microelectrodes filled with 3 M-KCl, one intracellular and the other extracellular placed closely together. The action potential was electronically differentiated with an R-C

circuit having a time constant of 35 μsec. The amplitude of the differentiated trace during the upstroke of the action potential gave a precise measure of the maximum rate of depolarization ($(dV/dt)max$) and was linear from 0·10 to 1000 V/sec. The records were photographed from the oscilloscope screen on 35 mm film. Action potential duration was measured from the rapid upstroke of the action potential to repolarization at 0 mV (plateau duration), and within 0·5 mV of the return to the resting potential. This latter point was considered to be the end of the action potential and was subject to a measurement error of not more than 5 msec.

A single 'test' stimulus of 5 msec duration was introduced between two conditioning action potentials by a second stimulator connected in series to the first and triggered by the conditioning stimulus. The interval between the conditioning and test stimuli was then progressively decreased (see Fig. 1) and the strength of the test stimulus adjusted so that the interval from the stimulus to the onset of the action potential remained constant. This adjustment was necessary because slight changes in this interval resulted in changes in $(dV/dt)max$. The test interval was defined as the interval between the completely (within 0·5 mV) repolarized conditioning action potential and the onset of the following test action potential.

In order to depolarize the fibre in a stepwise fashion from the original resting potential to approximately −60 mV, the potassium concentration of the perfusate in the proximal compartment was increased progressively to approximately 20 mM by adding small volumes of a 500 mM-KCl solution. In some experiments, the calcium concentration of the perfusate was increased to 7·2 mM. In other experiments, constant current pulses were introduced in order to vary action potential duration. In all but three of the experiments reported in this study the microelectrode was maintained in the same cell throughout the entire experimental protocol.

Voltage clamp experiments were performed in thin (0·4–0·8 mm) ventricular trabeculae from pig and sheep hearts using the three compartment chamber with the single sucrose-gap. In essence the method was the same as described by Beeler & Reuter (1970*a*) except that an improved voltage clamp amplifier was used.

RESULTS

Recovery of steady-state $(dV/dt)max$

Effect of membrane potential. Fig. 1 illustrates the experimental method and the principle results. The action potentials and associated differentiated upstrokes have been traced from the original records and remounted for greater clarity. The action potentials traced in continuous lines are the 'conditioning' action potentials recorded at a basic rate of 0·5/sec, those traced in broken lines are the 'test' action potentials recorded as the test interval was progressively decreased. The amplitude of the spikes in the differentiated traces shown below each action potential measures $(dV/dt)max$. The action potentials in the upper panel were recorded when the potassium concentration of the perfusate was 5·4 mM. The resting potential was −82 mV and the $(dV/dt)max$ of the conditioning action potentials, i.e. the steady-state $(dV/dt)max$, was 200 V/sec. The test intervals shown in the Figure are (from right to left) 190, 60, 35 and 20 msec respectively and the $(dV/dt)max$ of these test action potentials are 200, 200, 190 and 180 V/sec respectively. The second row of action potentials was recorded

when the potassium concentration in the perfusate was increased to 11 mM and the resting membrane potential had fallen to −68 mV. The steady-state (dV/dt)max has decreased to 128 V/sec. The test intervals are 220, 170, 65 and 30 msec and the (dV/dt)max of these test action potentials are 128, 120, 80 and 50 V/sec respectively. The results of these experiments, including those obtained when the resting membrane potential was depolarized to −76 mV, are shown in greater detail in Fig. 2. (dV/dt)max of the test action potentials is shown on the ordinate. The test interval is

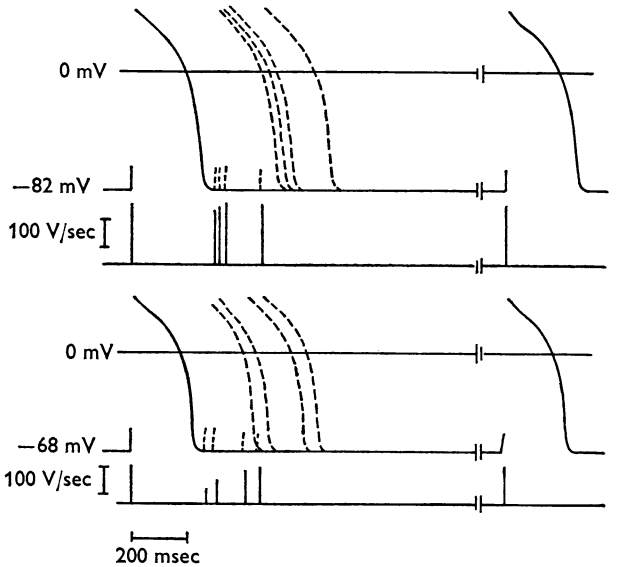


Fig. 1. Guinea-pig papillary muscle action potentials and corresponding differentiated upstrokes traced from original records from which measurements were made. Records were obtained during continuous impalement of the fibre. The action potentials traced in the continuous line are the conditioning action potentials recorded at a basic rate of 0·5/sec. Those traced in the interrupted lines are the test action potentials recorded as the test interval was progressively decreased. The action potentials in the lower panel were recorded after the resting potential had been decreased to −68 mV by the addition of KCl to the perfusate.

shown on the abscissa. At each level of resting membrane potential, the decrease in (dV/dt)max which occurred as the test interval became progressively shorter could be fitted by a single exponential. The time constants with which (dV/dt)max in the test action potentials regained the steady-state value, indicated by the arrows, were 18 msec when the resting potential was −82 mV, 28 msec when the resting potential was −76 mV and 70 msec when the resting potential was −68 mV. Similar experiments

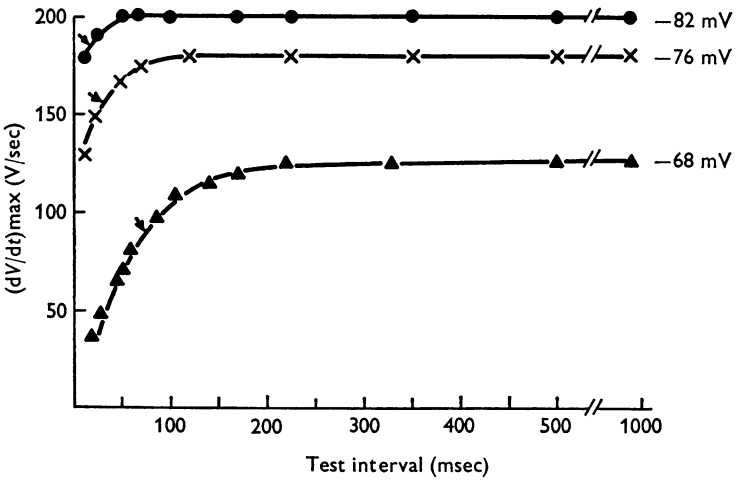


Fig. 2. Graphic representation of the data obtained in the experiment shown in Fig. 1, including results associated with a resting potential of −76 mV. The arrows indicate the time constants whereby (dV/dt)max in the test responses regained the steady-state value at the various levels of resting potential indicated beside each curve.

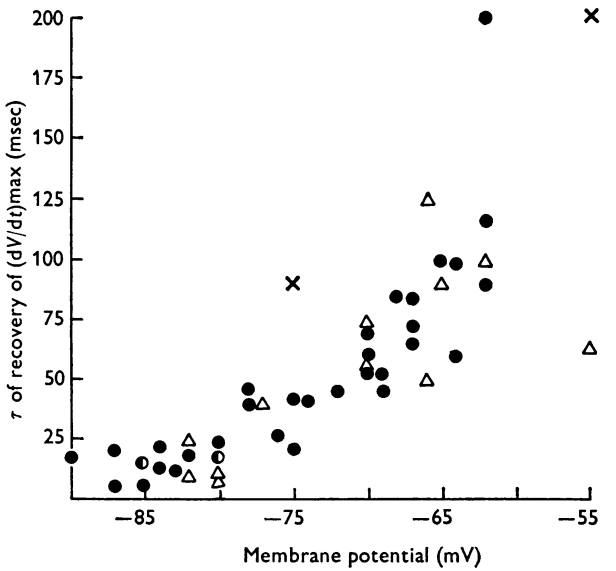


Fig. 3. Compiled data from eleven guinea-pig papillary muscles (filled circles), one sheep ventricular trabecula (half-filled circles), one pig ventricular trabecula (crosses) and Purkinje fibres from one calf and three sheep hearts (open triangles). The time constants (τ) of recovery of (dV/dt)max after a conditioning action potential are shown on the ordinate. The resting membrane potentials are shown on the abscissa.

were performed in papillary muscles from ten additional guinea-pig hearts, ventricular trabeculae from one sheep and one pig heart and Purkinje fibres from one calf and three sheep hearts. The results of all experiments are shown in Fig. 3. The time constant (τ) of the recovery of (dV/dt)max in the test responses (ordinate) is plotted as a function of the resting potential (abscissa). The results in the various species and fibre types were similar. The time constant was less than 25 msec at membrane potentials between -80 and -90 mV, and increased to between 100 and 200 msec when the fibre was depolarized to less than -65 mV.

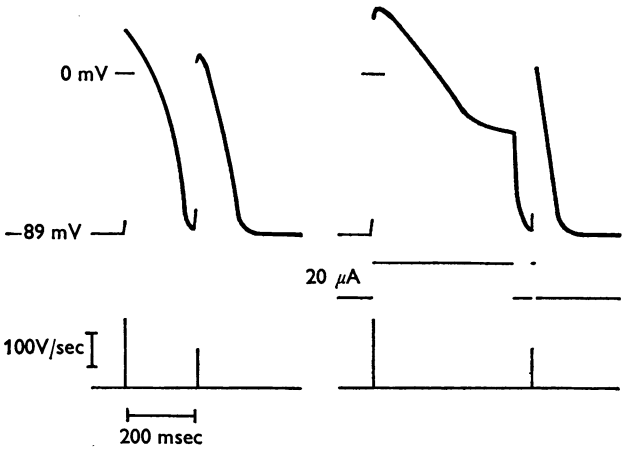


Fig. 4. Pairs of conditioning and test action potentials and the associated differentiated upstrokes from the proximal end of the guinea-pig papillary muscle. The records are traced from the original. The test interval in both pairs is approximately 0 msec. The pair of action potentials on the right was recorded during the injection of a constant current pulse of 20 μA strength and 430 msec duration into the muscle.

In order to assess the effects of changes in the duration of the conditioning action potential plateau on (dV/dt)max in the test action potential, we passed hyperpolarizing or depolarizing current through the sucrose-gap in to the proximal end of the fibre during the conditioning response. Fig. 4 illustrates such an experiment. Each pair of traced action potentials shows the conditioning and test potentials. The test intervals are equal and close to 0 msec. On the left, the duration of the conditioning action potential, measured at 0 and -30 mV, is 85 msec and 140 msec. On the right, a 430 msec constant current pulse of 20 μA strength has prolonged the duration of the conditioning action potential to 200 msec at 0 mV and to 430 msec at -30 mV. This change in the duration of the conditioning action potential has not affected (dV/dt)max in the test response. Nor did it alter the time constant of recovery of (dV/dt)max which in this

experiment was 20 msec. However, prolonging the duration of the conditioning action potential has shortened the duration of the test action potential probably because of the more complete inactivation of the slow inward calcium current during the prolonged conditioning action potential. In four other experiments, variations in the plateau duration of the conditioning action potential from 70 msec (hyperpolarizing pulses) to 500 msec (depolarizing pulses) were similarly without effect on the time constant of recovery of (dV/dt)max.

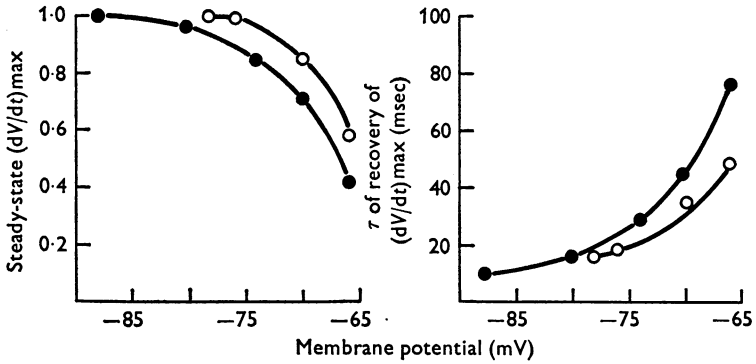


Fig. 5. Curves relating steady-state (dV/dt)max to resting membrane potential (left) and time constant (τ) of recovery of (dV/dt)max to resting membrane potential (right) at two different external calcium concentrations (1·8 mM, filled circles and 7·2 mM, open circles).

Effect of increasing external calcium concentration. It has been established previously (Weidmann, 1955*b*; Beeler & Reuter, 1970*a*), that an increase in external calcium concentration shifts the curve relating the steady-state rapid inward current or (dV/dt)max to membrane potential along the voltage axis in the depolarizing direction. We studied the effects of a fourfold increase (from 1·8 to 7·2 mM) in the calcium concentration of the perfusate in five guinea-pig papillary muscles and one sheep Purkinje fibre. Fig. 5 shows the results of one of the papillary muscle experiments. On the left, the relative steady-state (dV/dt)max is plotted against the membrane potential and on the right, the time constant of the recovery of the steady-state (dV/dt)max is plotted against membrane potential. The results indicated by the filled circles were obtained when the calcium concentration in the perfusate was 1·8 mM and those indicated by the open circles, when the calcium concentration was 7·2 mM. The increase in external calcium is associated with a 3 mV shift of both curves along the voltage axis in the depolarizing direction. The results of all experiments are shown in Table 1. A complete experiment, similar to that shown in Fig. 5, was performed in one other guinea-pig papillary muscle and in one Purkinje

fibre. In both, the fourfold increase in external calcium concentration shifted the curves shown in Fig. 5 by 4 mV in the depolarizing direction. The less complete data obtained in the remaining papillary muscle experiments were consistent with these results.

TABLE 1. Effect of increasing external Ca concentration on steady-state (dV/dt)max and recovery of (dV/dt)max

		$[Ca]_o$ = 1·8 mM			$[Ca]_o$ = 7·2 mM		
Expt. no.	Tissue	Membrane potential (mV)	Steady-state (dV/dt)max (V/sec)	τ recovery (dV/dt)max (msec)	Membrane potential (mV)	Steady-state (dV/dt)max (V/sec)	τ recovery (dV/dt)max (msec)
1	pm	−80	240	20	−78	240	18
		−75	230	42	−76	240	23
		−70	190	53	−70	205	48
		−65	110	98	−66	150	72
2	pm	−78	190	37	−78	190	31
		−72	132	45	−72	176	35
		−68	108	87	−68	148	40
		−62	28	210	−62	100	85
3	pm	−67	40	83	−68	116	19
4	pm	−77	130	32	−77	160	19
5	pm	−64	96	60	−64	130	54
6	Pf	−82	420	25	−82	500	15
		−70	306	73	−72	390	49
		−66	132	125	−65	260	68

τ, time constant. pm, papillary muscle. Pf, Purkinje fibre.

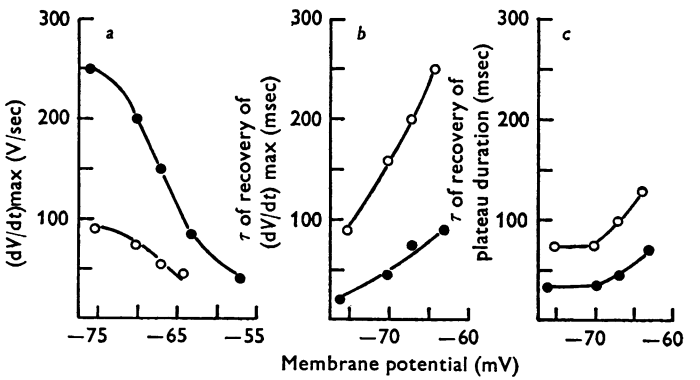


Fig. 6. Effects of 10° C change in temperature on curves relating steady-state (dV/dt)max (left), time constant (τ) of recovery of (dV/dt)max (middle) and time constant (τ) of recovery of plateau duration (right) to resting membrane potential. Values obtained at 37° C are shown as filled circles, and those obtained at 27° C are shown as open circles.

Effect of temperature. The temperature dependence of the steady-state relationships and recovery kinetics of (dV/dt)max were studied in three guinea-pig papillary muscles. The results from one of these experiments are shown in Fig. 6. The results shown by the filled circles were obtained when the temperature of the perfusate was 37° C. Those shown by the open circles were obtained at 27° C. The steady-state relationships between (dV/dt)max and membrane potential are plotted in Fig. 6*a*. The temperature coefficient, Q_{10}, ranged between 2·0 and 2·8 within the membrane potential range of -75 to -64 mV. In two other experiments, Q_{10} ranged between 1·8 and 2·5 within the potential range of -86 to -58 mV. In these experiments the membrane potential at which complete inactivation of the sodium system occurred was not significantly altered by the temperature change. The relationship between the time constant of the recovery of (dV/dt)max and membrane potential are plotted in Fig. 6*b*. The Q_{10} ranged between 3·0 and 3·7. In one other experiment, Q_{10} ranged between 2·8 and 4·2 within the potential range of -84 to -65 mV. While the change of the steady-state curve in Fig. 6*a* is a measure of the temperature sensitivity of the maximal sodium conductance ($\bar{g}_{Na}$), the Q_{10} of the time constants (Fig. 6*b*) indicates the temperature dependence of the kinetics in the sodium channels. The relationship between the time constant of the recovery of the plateau duration and membrane potential are plotted in Fig. 6*c* and will be discussed below.

(dV/dt)max in test responses arising from incompletely repolarized fibres. The results described in the preceding sections would predict that (dV/dt)max of test responses arising from incompletely repolarized fibres and therefore from a decreased membrane potential would be: (1) less than the steady-state (dV/dt)max associated with that level of membrane potential but (2) similar to the (dV/dt)max of a test response arising from that level of membrane potential when the test interval was 0 msec. These predictions are confirmed in the experiments shown in Figs. 7 and 8. In Fig. 7, the (dV/dt)max of test responses arising progressively earlier from an incompletely repolarized fibre whose resting potential was -87 mV are shown in the open circles. The steady-state values of (dV/dt)max, recorded as the same fibre was depolarized by the addition of KCl to the perfusate, are shown in the filled triangles. The (dV/dt)max of test responses arising from the incompletely repolarized fibre, i.e. from membrane potentials less negative than the resting potential, are much less than those of steady-state responses arising from similar membrane potential levels. The membrane potential associated with a 50 % reduction in the (dV/dt)max was -79 mV in the test responses arising from the incompletely repolarized fibre and -66 mV in the steady-state response. Thus, the (dV/dt)max of action potentials arising from an incompletely

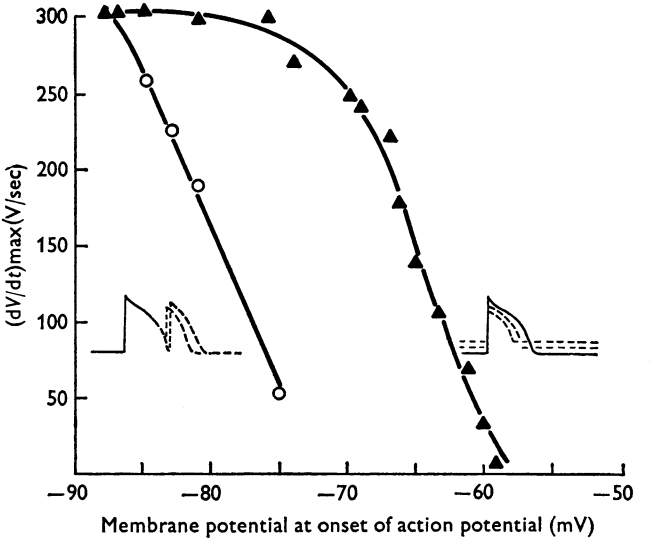


Fig. 7. Curves relating (dV/dt)max to resting membrane potential. The values indicated by the filled triangles are steady-state values, obtained when the guinea-pig papillary muscle, stimulated at a rate of 0·25/sec, was depolarized by adding KCl to the perfusate (insert right). The values indicated by the open circles are non-steady-state values, obtained in the same fibre from test responses initiated during the phase of incomplete repolarization of the preceding conditioning response (insert left).

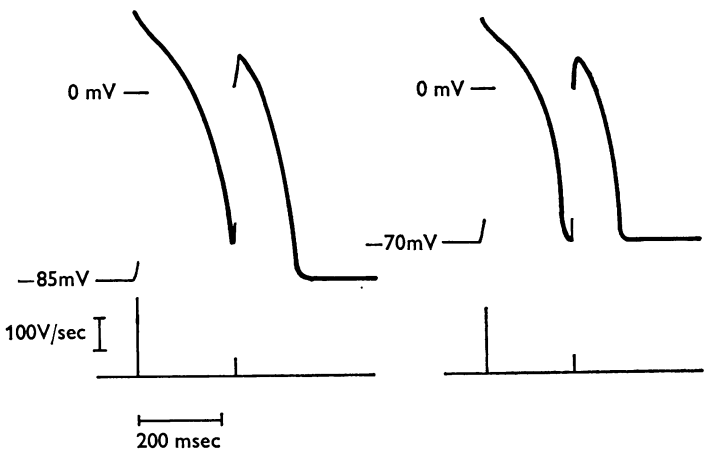


Fig. 8. Pairs of conditioning and test action potentials and the associated differentiated upstrokes traced from original records obtained in guinea-pig papillary muscle. In the pair on the left, the test action potential arises before the preceding conditioning action potential has returned to its resting value. The membrane potential at the onset of the test action potentials is −70 mV. In the pair on the right, the same fibre has been depolarized to −70 mV by the addition of KCl to the perfusate. The onset of the test action potential coincides with the return of the conditioning action potential to its resting level, and therefore with a test interval of approximately 0 msec.

repolarized fibre was shifted 13 mV along the voltage axis in the hyperpolarizing direction as compared to the steady-state $(dV/dt)max$. In four similar experiments, the shifts of these curves along the voltage axis were 10, 10, 11 and 11 mV.

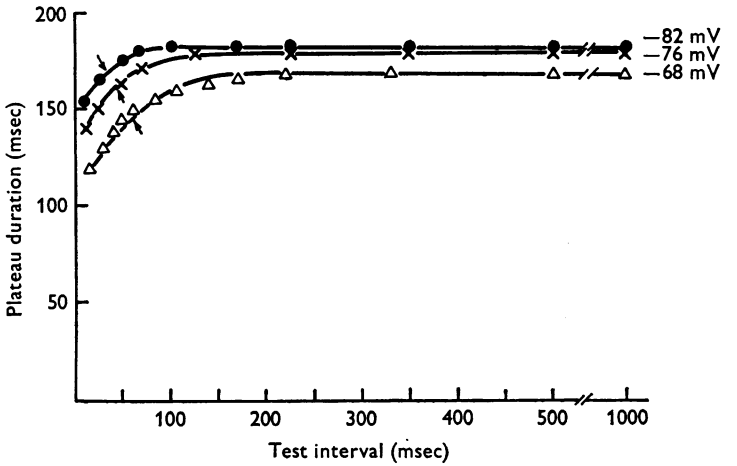


Fig. 9. Graphic representation of the data obtained in the experiment shown in Fig. 1 including results associated with a resting potential of −76 mV. The arrows indicate the time constants with which the plateau duration, i.e. the duration of the action potential at 0 mV in the test responses, regained the steady-state value at the various levels of resting potential indicated beside each curve.

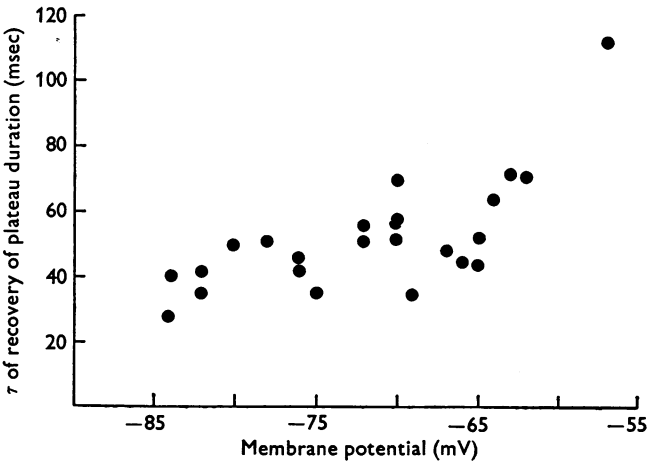


Fig. 10. Compiled data from eleven guinea-pig papillary muscle experiments. The time constants (τ) of the recovery of the plateau duration are shown on the ordinate. The resting membrane potentials are shown on the abscissa.

The pairs of conditioning and test action potentials traced in Fig. 8 confirm the second prediction. On the left, the resting potential is −85 mV and the steady-state (dV/dt)max is 240 V/sec. The test response occurs before the fibre is completely repolarized and originates from a potential of −70 mV. The (dV/dt)max of this test response is 50 V/sec. On the right, the fibre has been depolarized to −70 mV by the addition of KCl to the perfusate and steady-state (dV/dt)max has decreased to 200 V/sec. This decrease is anticipated from the curve relating steady-state (dV/dt)max and membrane potential exemplified by the filled triangles in Fig. 7. The test response occurs as soon as the fibre has been completely repolarized, i.e. following a test interval of 0 msec. Its (dV/dt)max, as in the test response on the left, is 50 V/sec.

Recovery of steady-state plateau duration

Effect of membrane potential. The plateau duration of the test responses, i.e. the duration of the action potential at 0 mV, was, like (dV/dt)max, analysed as a function of time and membrane potential. The results of such an analysis are presented in Fig. 9. The experiment from which these data were obtained is shown in Fig. 1. When the resting potential was −82 mV, the plateau duration in the test responses did not decrease until the test interval had decreased to less than 100 msec. This result is similar to that previously described in the ventricular myocardial fibres of the pig moderator band (Gettes *et al.* 1972). As the test interval was made progressively shorter than 100 msec, the plateau duration progressively decreased. This decrease, like the decrease in (dV/dt)max, could be fitted by a single exponential. The time constant whereby the plateau duration in the test responses regained the steady-state value (arrow) was 30 msec. When the fibre was depolarized to −76 mV and then to −68 mV, the time constant of the recovery of the steady-state value increased to 45 msec and then to 60 msec. The compiled results of the guinea-pig papillary muscle experiments are shown in Fig. 10. The Figure shows that the time constant of the recovery of the steady-state plateau duration increased within the range of 30–70 msec as the membrane potential was changed from −85 to −60 mV. In the potential range of −85 to −80 mV the recovery of the plateau duration was significantly ($P < 0{\cdot}005$) slower than the recovery of the (dV/dt)max but became faster ($P < 0{\cdot}01$) in the potential range of −65 to −60 mV.

Effect of temperature. The effects of a 10° C change in temperature on the time constant of the recovery of the steady-state plateau duration is shown in Fig. 6*c*. When the temperature was 37° C the time constant, in this experiment, increased from 30 msec at a membrane potential of −76 mV to 70 msec as the membrane potential decreased to −62 mV.

When the temperature was 27° C, the time constant increased from 70 to 120 msec as the membrane potential underwent a similar decrease. Thus, the Q_{10} of the plateau recovery kinetics in this experiment ranged from 1·7 to 2·3. In a second similar experiment, the Q_{10} ranged from 1·5 to 1·9 within the potential range of −84 to −65 mV. These temperature coefficients for ventricular myocardial action potential plateau are considerably less than those obtained for the plateau of Purkinje fibres by Coraboeuf & Weidmann (1954). This may indicate that different mechanisms are involved in the action potential plateaus of the two fibre types (Gettes *et al.* 1972; Hauswirth, Noble & Tsien, 1972).

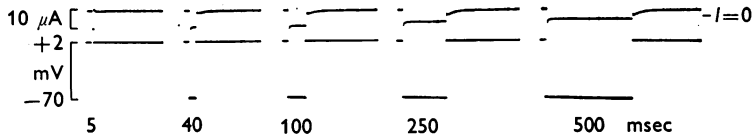


Fig. 11. Records from a voltage clamp experiment performed in a sheep ventricular trabecula which was bathed in Tyrode solution containing tetrodotoxin 2×10^{-5} g/ml. The current trace (top row) shows the recovery of the slow inward current after complete inactivation at +2 mV. Recovery was induced by repolarizing voltage clamp steps to −70 mV (lower row).

Voltage clamp experiments. Fig. 11 illustrates a voltage clamp experiment performed to determine the recovery kinetics of the slow inward calcium current. A sheep ventricular trabecula was constantly depolarized to +2 mV (holding potential) in Tyrode solution containing tetrodotoxin, 2×10^{-5} g/ml. Under these conditions the calcium current is inactivated by the positive holding potential and the sodium current is eliminated by tetrodotoxin. At a rate of 0·3/sec the membrane was repolarized from the holding potential to −70 mV by clamp steps of progressively longer duration. As can be seen in Fig. 11 the slow inward current progressively increased in amplitude when the potential was clamped back to +2 mV and regained its steady-state value when the duration of repolarization to −70 mV was between 100 and 250 msec.

Figs. 12 and 13 show the complete results from an experiment on a pig ventricular trabecula. The conditions of this experiment were identical to those of the experiment illustrated in Fig. 11. In Fig. 12 the amplitude of the slow inward current during depolarization to +2 mV is plotted against the duration of the repolarizing clamp steps to four different potential levels. The time course whereby the slow inward current (I_{Ca}) regained the steady-state at each potential level can be fitted by exponentials. The time constants, shown by the arrows in Fig. 12, increased from 60 msec when the membrane potential during repolarization was

clamped to −69 mV to 250 msec when it was clamped to −9 mV. In Fig. 13 the relationship between the steady-state recovery from inactivation of the slow inward current and membrane potential are shown on the left. This relation extends to much more positive potentials than does a similar plot of steady-state (dV/dt)max. At potentials where steady-state (dV/dt)max is zero (Fig. 7) the slow inward current can still be fully activated. On the right of Fig. 13, the time constants of recovery of the

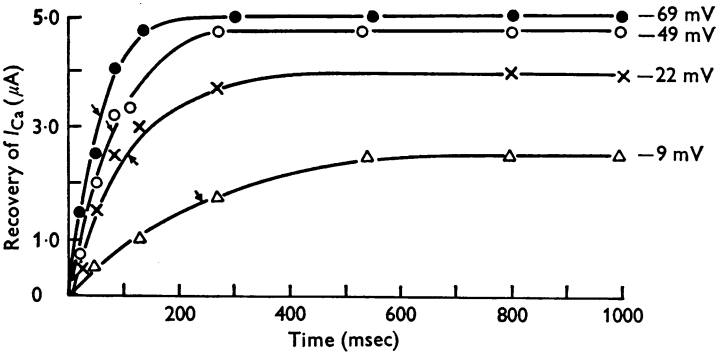


Fig. 12. Relationship between recovery of inward calcium current (I_{Ca}) and duration of repolarizing clamp steps from +2 mV to different potential levels. The respective potential levels of repolarization are indicated beside each curve. The curves are drawn as exponentials, the arrows indicate the respective time constants. Experiment performed in a pig ventricular trabecula in Tyrode solution containing tetrodotoxin 2×10^{-5} g/ml.

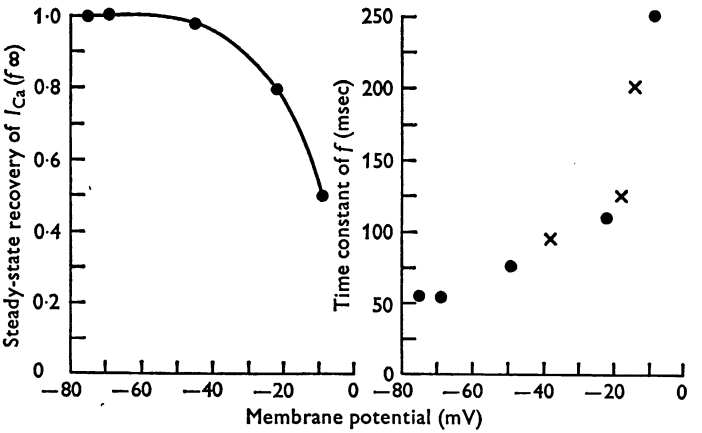


Fig. 13. Left: steady-state relation between recovery of inward calcium current from inactivation and membrane potential; right: relationship between time constants of recovery from inactivation (filled circles) or time constants of inactivation (crosses) and membrane potential. Same experiment as in Fig. 12.

slow inward current from inactivation (filled circles) are plotted against membrane potential. This plot also includes time constants of inactivation of the slow inward current (crosses), measured in the same preparation from the exponential decay of this current at -38, -17 and -10 mV. The fact that all of the experimental points fall on the same line strongly suggests that inactivation and the recovery from inactivation of the slow inward current are identical. Furthermore, this and three similar experiments showed that in the potential range -80 to -60 mV both the time constants of recovery of the slow inward current and the time constants of recovery of plateau duration (Fig. 10) were between 30 and 70 msec. These results suggest that the changes in plateau duration shown in Figs. 1, 4 and 9 can be attributed to the time course of recovery of the inward calcium current from partial inactivation induced during the conditioning action potential.

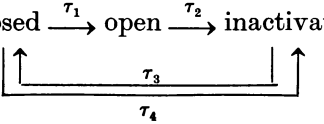
DISCUSSION

The main results of our study can be summarized as follows: (1) the time constants for reactivation of the inward sodium current responsible for (dV/dt)max is much slower than anticipated from earlier experiments in cardiac Purkinje fibres (Weidmann, 1955*a*) and in nerve (Hodgkin & Huxley, 1952), (2) the kinetics of the current systems responsible for the rapid upstroke and for the plateau of the ventricular myocardial action potential are different, although both are voltage dependent, and (3) the time constants for the plateau to regain its steady-state value correspond to the time constants for reactivation of the slow inward calcium current.

Quantitative study of the rapid inward sodium current in cardiac muscle by the voltage clamp method is much less reliable than in the squid axon because of the morphology of the preparations and the difficulty in achieving rapid uniform changes of transmembrane voltage (Beeler & Reuter, 1970*a*; Giebisch & Weidmann, 1971; Kootsey & Johnson, 1972). However, (dV/dt)max of the upstroke of the action potential has been accepted as a valid index of the inward sodium current since Hodgkin's & Katz's (1949) work on squid axon and since it was shown in cardiac muscle that (dV/dt)max varied in direct proportion to changes in the external sodium concentration (Weidmann, 1955*a*; Brady & Woodbury, 1960). Furthermore, Weidmann (1955*a*) showed that the steady-state curve relating (dV/dt)max to membrane potential in Purkinje fibre was similar to the steady-state curve relating rapid inward sodium current to membrane potential in the squid axon. In our experiments we altered the membrane potential by changing the potassium concentration of the perfusate rather than by injecting current into the preparation. In this way, problems related to non-uniformity of voltage control and electrical artifacts were avoided. Never-

theless, the steady-state curve relating (dV/dt)max to changes in membrane potential which we have recorded were similar to those observed by Beeler & Reuter (1970*a*) and by New & Trautwein (1972) who studied the inward sodium current in mammalian papillary muscles by the voltage clamp technique utilizing a single sucrose-gap. These results indicate that the steady-state changes in inward sodium current and (dV/dt)max were similar regardless of the method used to change the membrane potential.

It was first shown by Hodgkin & Huxley (1952) that when a squid axon membrane was subjected to a 44 mV depolarization, inward current increased rapidly to a maximum and then declined (was inactivated) with a time constant of 1–2 msec. The magnitude of the current depended on the membrane potential prior to the sudden 44 mV depolarization and decreased as this membrane potential became less negative. The time constant with which the sodium current reached a steady-state was determined by the voltage of the conditioning depolarizing or hyperpolarizing clamp step and did not exceed 15 msec. Utilizing a 2-pulse method, they showed that recovery of the inward current system from inactivation also occurred with a time constant of less than 15 msec. As a result of these observations, they proposed the following reaction scheme for the kinetics of the Na channels in squid axon:



where the time constants τ_2, τ_3, and τ_4 are identical. Beeler & Reuter (1970*a*) and New & Trautwein (1972) showed that in mammalian ventricular fibres the time constant of inactivation of the rapid inward current system after a sudden depolarization was shorter than 5 msec and hence similar to the squid axon. However, insufficient voltage control during the flow of inward sodium current precluded detailed kinetic studies. Also the recovery of the rapid sodium current from inactivation has not been studied extensively in mammalian cardiac fibres. Weidmann (1955*a*) observed that in sheep Purkinje fibres, the time constant of recovery of (dV/dt)max after a preceding depolarization was less than 20 msec when the membrane potential prior to depolarization ranged between -80 and -106 mV. From this result, which agrees essentially with our data at this potential range, he concluded that both inactivation of the sodium current and its recovery from inactivation are very rapid processes. However, Weidmann did not study the recovery of (dV/dt)max at membrane potentials less negative than -80 mV. Our results show that in both ventricular myocardial and Purkinje fibres, the time constant of recovery of (dV/dt)max becomes progressively prolonged as the mem-

brane is depolarized to potentials less negative than -80 mV and may exceed 100 msec when the resting potential is in the range of -65 to -60 mV. This is more than one order of magnitude slower than the time constant of inactivation of the sodium current. Haas *et al.* (1971) showed in frog atrial fibres that at lower temperatures the recovery of the rapid inward current following a preceding depolarization exceeded the time constant of inactivation of the current by a factor of 50. Their and our observations indicate that in cardiac muscle, the recovery of the rapid inward current system from inactivation is not identical with the rate of inactivation of the system as it is in squid axon.

Several mechanisms may be postulated to explain the decrease in (dV/dt)max which we observed in early test responses. These include (1) a persistent effect of outward current generated during the preceding plateau, (2) accumulation of intracellular sodium causing a shift of the sodium equilibrium potential and a subsequent decrease in the driving force for sodium current, and (3) a change in the kinetic variables determining the time course of conductance changes. The outward current hypothesis can be excluded by the fact that time dependent outward currents, if present at all in mammalian ventricular myocardial fibres, are very small (Giebisch & Weidmann, 1971). In addition, changes in plateau duration (Fig. 4) of the conditioning action potential did not alter the recovery time of (dV/dt)max in the test response. The possibility of sodium accumulation is also very unlikely. This explanation would not be consistent with our observation that recovery is fastest when the action potential arises from the most negative potentials, since, at this condition, the inward sodium current is largest and sodium accumulation should be greatest.

The following observations suggest that changes in sodium conductance were primarily responsible for the decrease in (dV/dt)max in the test response: (*a*) a fourfold increase in extracellular calcium shifted the curve relating the time constant of recovery of (dV/dt)max in the test response to membrane potential along the voltage axis by the same amount as it shifted the steady-state (dV/dt)max membrane potential curve. This is consistent with the well known effect of change in membrane surface charge on membrane conductance (Frankenhaeuser & Hodgkin, 1957). (*b*) The time constants with which (dV/dt)max adjusted to a new steady-state value following subthreshold voltage clamps (i.e. development of inactivation without opening of the sodium channel) were similar to the time constants with which (dV/dt)max regained the steady-state value after a preceding action potential (L. S. Gettes & H. Reuter, unpublished results).

On the basis of our results, we suggest that the reaction model of the

sodium channels in the squid axon (see above) may be modified as follows for cardiac muscle: τ_3 and τ_4 are equal, but much slower than τ_2. τ_1 and τ_2 are rapid and may be similar to those in squid axon. This may indicate that a large fraction of the sodium channels has to be opened simultaneously in order to be rapidly inactivated. In contrast, the time constants of inactivation and of recovery from inactivation of the slow inward calcium current were identical, even after the conductance channels were open. Hence the reaction scheme in which τ_2, τ_3 and τ_4 are identical may be applied to this slow conductance system.

The changes in plateau duration observed in this study are in agreement with the results previously obtained in the pig moderator band (Gettes *et al.* 1972). In that study, the duration of the premature action potential decreased as the preceding diastolic interval became shorter than 100 msec. The similarity between the time constant of recovery of the plateau duration and the time constant of recovery of the slow inward current suggests that in ventricular myocardial fibres, the kinetics of the slow inward current can account for the plateau duration in premature responses. The results from our study suggest therefore, that the plateau duration in a premature response will depend on both the resting membrane potential and the preceding diastolic interval. In contrast to our results in ventricular myocardial fibres are those of Hauswirth *et al.* (1972) who showed that in Purkinje fibres, the kinetics of outward currents were the primary determinants of premature action potential duration. Thus, our observations provide an additional piece of evidence linking the plateau of the ventricular myocardial action potential to the calcium-dependent slow inward current rather than to the slowly increasing potassium outward current (Beeler & Reuter, 1970*a*; Giebisch & Weidmann, 1971; New & Trautwein, 1972).

Further implications of our data may be listed as follows: (1) (dV/dt)max in premature beats will depend on the diastolic interval preceding the premature action potential as well as on the membrane potential at the onset of the premature response, (2) the action of antiarrhythmic drugs may be due to alterations in kinetics as well as, or perhaps in lieu of, changes in steady-state characteristics of the rapid inward current system.

(1) *Premature responses*

Our study indicates that (dV/dt)max in premature responses arising from incompletely repolarized fibres will be less than the steady-state (dV/dt)max of the action potentials arising from a similarly decreased resting membrane potential. The dual control of (dV/dt)max, i.e. its voltage and time dependence, may be of particular importance when only

a portion of the myocardium is partially depolarized such as occurs following coronary artery occlusion. It has been shown that the occlusion of a coronary artery results in an increase in extracellular potassium (Harris, 1966) and a fall in resting potential (Kardesch, Hogancamp & Bing, 1958; Taggart & Slater, 1971) in the ischaemic zone. These changes will have two effects: (1) (dV/dt)max in the non-premature response will be slower in the ischaemic than in the surrounding non-ischaemic zone because of the effect of the lowered resting membrane potential, (2) the differences between (dV/dt)max in ischaemic and non-ischaemic zones in an earlier premature response will be even greater than in a non-premature response because of the prolonged reactivation time associated with the fall in resting potential in the ischaemic zone. These changes may cause inhomogeneities of conduction and may result in the development of abnormal conduction pathways and unidirectional blocks, thereby contributing to the development of reentrant arrhythmias.

(2) *Antiarrhythmic drug effects*

Most clinically effective antiarrhythmic drugs have in common the ability to alter the relationship between the membrane potential and (dV/dt)max (ref. in Gettes, 1971). In Weidmann's (1955*b*) original description of this effect, the fibre was stimulated at a rate of 1/sec and membrane potential was changed by a modified voltage clamp. Since then, most studies have been performed by stimulating the fibre during its phase of incomplete repolarization. Thus, these studies do not permit a separation of drug induced changes in 'membrane responsiveness' into those resulting from changes in steady-state characteristics and those resulting from changes in kinetic characteristics of (dV/dt)max. Johnson & McKinnon (1957) showed that quinidine caused almost no change in (dV/dt)max of rabbit ventricular fibres when the driving rate was 0·1/sec, but caused a progressive decrease in (dV/dt)max as driving rate was increased. Heistracher (1971) studied (dV/dt)max in quinidine-treated guinea-pig fibres and found that the (dV/dt)max in the first response following a quiescent period of several minutes was unchanged from control. With each subsequent action potential, (dV/dt)max decreased until it reached a new steady state. These studies suggest that the quinidine-induced decrease in (dV/dt)max is rate rather than voltage dependent. A similar rate dependency has been observed for other antiarrhythmic drugs (Tritthart, Fleckenstein & Fleckenstein, 1971), and for phenothiazines (Arita & Surawicz, 1972). It is possible that a distinction of drug effects by their influence on steady-state or kinetic parameters would permit a more rational classification of the antiarrhythmic drugs and will aid in the understanding of both their beneficial and adverse clinical effects.

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