Effects of External Calcium, Calcium Channel-Blocking Agents, and Stimulation Frequency on Cycle Length-Dependent Changes in Canine Cardiac Action Potential Duration

THOMAS J. COLATSKY AND PERRY M. HOGAN

SUMMARY Action potentials were recorded from canine Purkinje fibers to evaluate the effects of external calcium and the slow inward current-blocking agents, manganese and verapamil, on the relationship between action potential duration and diastolic interval during both steady state and premature excitation. Elevated external calcium shortened the action potential without changing the dependence of either steady state or premature responses on the preceding diastolic interval. The effects of elevated calcium resembled those of increased stimulation frequencies in that the duration of the steady state action potential was reduced without a concomitant change in the interval dependence of the premature action potentials. In contrast, manganese and verapamil markedly slowed the recovery of the premature action potential duration with increasing diastolic interval, but did not significantly alter the steady state relationship. On the basis of these experiments, we conclude that (1) the mechanisms underlying the immediate and cumulative cycle length-dependent changes in action potential duration are separate processes; (2) the abrupt decrease in action potential duration following a reduction in cycle length is consistent with the incomplete recovery of the plateau currents, whereas the steady state changes involve a slower process, possibly an increase in potassium conductance mediated by an accumulation of internal calcium; and (3) the slower frequency-dependent changes do not depend on calcium entry through the slow inward current channel.


THE cardiac action potential is quite sensitive to changes in cycle length (Hoffman and Cranefield, 1960; Carmeliet, 1977). Previous studies have shown that an action potential initiated prematurely during diastole generally differs from the preceding steady state response in both duration and waveform. In ventricular Purkinje fibers, a sudden reduction in cycle length shortens the action potential by decreasing the plateau duration with relatively little effect on the time course of late repolarization (Vick, 1971; Gettes et al., 1972; Hauswirth et al., 1972). The response in atrial and ventricular muscle cells is usually more complex, involving an initial elevation and prolongation of the plateau phase and a marked steepening of the final repolarization limb (Edmands et al., 1966; Greenspan et al., 1967; Miller et al., 1971; Gettes et al., 1972).

The cycle length-dependence of cardiac action potential duration generally has been attributed to the incomplete recovery of ionic currents activated during the plateau phase of the preceding action potential (Noble and Tsien, 1969b; Vick, 1971; Hauswirth et al., 1972; McAllister et al., 1975; Beeler and Reuter, 1977), although alternative hypotheses also exist (Hoffman and Cranefield, 1960; Gibbs et al., 1963; Bass, 1975; Bassingthwaighte et al., 1976; Kline and Morad, 1976; Carmeliet, 1977). Hauswirth et al. (1972) found a close correspondence in sheep Purkinje fibers between the progressive recovery of action potential duration with increasing diastolic interval (called the interval-duration relation) and the deactivation time course of the delayed outward current, \( i_{\text{K}} \). A similar correlation was made in ventricular muscle fibers between the degree of reaction of the slow inward current, \( i_{\text{si}} \), and the duration of the plateau phase in premature responses (Gettes and Reuter, 1974).

It has been proposed (Vick, 1971; Hauswirth et al., 1972) that these residual conductances may cumulate during repetitive stimulation to shorten the action potential in the steady state as well. However, several observations do not fit this scheme. For example, the development of a steady state in action potential duration with repetitive stimulation duration following a reduction in cycle length is consistent with the incomplete recovery of the plateau currents, whereas the steady state changes involve a slower process, possibly an increase in potassium conductance mediated by an accumulation of internal calcium; and (3) the slower frequency-dependent changes do not depend on calcium entry through the slow inward current channel.

return to slower rates of drive (see references in Carmeliet, 1977). One hypothesis consistent with these data is that stimulation at rapid frequencies shortens the cardiac action potential through an increase in potassium permeability which is mediated by calcium accumulation near the inner surface of the sarcolemma (Isenberg, 1975, 1977; Bassingthwaite et al., 1976). In ventricular cells, the frequency-dependent increase in outward current can be reduced by application of the calcium channel-blocking agents, verapamil and D600 (Bassingthwaite et al., 1976), suggesting that these changes depend on calcium entry via the slow inward current channel. These agents also appear to suppress $i_x$ in Purkinje fibers (Kass and Tsien, 1975), although such effects can be dissociated from changes in slow inward current.

In the present study, we have examined the effects of changes in calcium concentration and the slow inward current-blocking agents, manganese and verapamil, on the cycle length dependence of action potential duration in isolated canine Purkinje fibers, using both steady state and extrasystolic patterns of stimulation. Our purpose was (1) to determine whether these changes alter the restitution of action potential duration in premature responses (i.e., the interval-duration relation) in a way that is consistent with their reported effects on the plateau currents, and (2) to determine to what extent such alterations are reflected in the steady state relationship between action potential duration and diastolic interval. Preliminary reports of this work have appeared previously (Colatsky and Hogan, 1975; Colatsky and Hogan, 1976).

**Methods**

Bundles of Purkinje fibers with attached pieces of myocardium were dissected from either ventricle of hearts removed from adult mongrel dogs previously anesthetized with sodium pentobarbital (30 mg/kg, iv). Preparations were pinned to the Sylgard-covered bottom of a 10-ml Plexiglas chamber and superfused with oxygenated Tyrode’s solution at a rate of about 10 ml/min. The pH and temperature were held constant during each experiment (pH range, 7.2-7.3; temperature, 34.5-37°C).

The composition of the Tyrode’s solution was (mm): NaCl, 137, KCl, 2.7, CaCl$_2$, 1.8; NaHCO$_3$, 12.5; MgCl$_2$, 0.5; dextrose, 5.5; equilibrated with 98% O$_2$, 2% CO$_2$ gas mixture. Solutions containing 5.4 or 7.2 mM calcium or 3.6 mM manganese were made by adding appropriate amounts of the chloride salt of each substance to the standard Tyrode’s solution, with no compensation for changes in osmolality. Verapamil (Knoll Pharmaceutical Co.) was added from a stock solution prepared by dissolving 25 mg of crystalline racemic verapamil HCl in 100 ml of doubly distilled water to obtain final bath concentrations of 0.5-6.0 mg/liter.

Action potentials were recorded with intracellular glass microelectrodes containing 3 M KCl and having resistances of 5-15 MΩ. Action potential duration, measured from photographic records of oscilloscope tracings, was determined as the time required to repolarize to —25 mV, to —60 mV, and to 95% of the maximum diastolic potential, thus giving estimates of plateau duration, effective refractory period, and total action potential duration, respectively.

Two patterns of stimulation were employed to distinguish between the effects of steady state stimulation frequency per se and the instantaneous effects of diastolic interval on action potential duration. In both cases, just suprathreshold cathodal stimuli were delivered to the endocardial surface of the preparation through a capillary glass electrode filled with Tyrode’s solution. In the first of these procedures, repetitive stimuli were applied for 1-minute intervals at constant cycle lengths ranging from 0.25 to 10.0 seconds, i.e., stimulation frequencies of 6-240 pulses min. Although a true steady state required 2-5 minutes at each stimulation frequency, the changes in action potential duration appeared to be largely complete after 1 minute at each new frequency. Thus, the use of 1-minute stimulation intervals expedited each trial without introducing significant errors into the relationship between action potential duration and basic stimulation frequency. More important, complications in the analysis arising from frequency-induced alterations in diastolic membrane potential or prolonged exposure to a given experimental solution were avoided. Records were taken at the end of each interval just before changing to a new frequency. For convenience, the duration measured during steady state stimulation will be referred to as “standard duration.” In the second procedure, paired stimuli were used to determine the immediate effect of changing diastolic interval on the duration of the extrasystolic response. Extrasystolic stimuli were interposed with variable delay during the diastolic interval of every tenth normal steady state cycle. The duration of the extrasystolic response will be referred to as “test duration.” For each steady state frequency, data were obtained relating the duration of the extrasystolic response to the immediately preceding diastolic interval. In this manner, the interval-duration relation for each cell could be determined for basic stimulation frequencies ranging from 6 to 95 pulses/min.

Certain conventions, as outlined by Hauswirth et al. (1972), were followed in defining the parameters of the interval-duration analysis. Action potential duration and preceding diastolic interval were defined relative to —60 mV. Diastolic interval was defined as that time for which membrane potential remained negative to —25 mV, to —60 mV, and to 95% of the maximum diastolic potential, thus giving estimates of plateau duration, effective refractory period, and total action potential duration, respectively. As Hauswirth et al. (1972) pointed out, the use of —60 mV as a boundary between action potential duration and diastolic interval is reasonable since (1)
the major frequency-induced changes in action potential duration occur at potentials positive to $-60$ (i.e., changes in frequency have little or no effect on the time course of terminal repolarization, that is, repolarization from $-60$ mV to the maximum diastolic potential) and (2) action potential duration is insensitive to changes in take-off potential negative to $-60$ mV (Arita and Surawicz, 1973). In the present investigation, an additional rationale for following this procedure was to facilitate the comparison of our findings with those of previous reports, including voltage clamp measurements of repolarization currents. If the kinetic variables governing the plateau currents are of the Hodgkin-Huxley type (McAllister et al., 1975), the rate of decay or recovery of the currents should depend only on the diastolic potential and should be independent of the degree of activation. Therefore, changes in the height of the plateau, such as occur in the presence of manganese and verapamil (cf., Fig. 8), should be relatively unimportant in determining the shape of the interval-duration relation, but may, however, influence the absolute duration of the responses recorded (Kass and Tsien, 1976). In this study, only extrasystolic responses originating from a fully repolarized diastolic potential were included in the analysis. Furthermore, cells were rejected if diastolic membrane potential varied more than 3 mV during the course of the trial.

When the interval-duration relation is expressed graphically, the curve generally shows two phases: an initial rapidly changing component followed by a slower component. The two components can be described as exponential processes. The time constants for these components were determined from semilog plots of the data (see Results, Fig. 4).

Experimental results are presented graphically in both absolute and normalized form. Normalization procedures are described in the appropriate sections of the Results.

Each experiment was preceded by at least 1 hour of equilibration in normal (1.8 mM calcium) Tyrode’s solution at a stimulation frequency of 95 pulses/min. A 15-minute equilibration period followed each subsequent solution change. Microelectrode impalement was maintained continuously throughout each experiment. Therefore, the results described in the paper represent paired observations. Data reduction and analysis including Student’s paired $t$-test were performed on a laboratory computer (PDP 8/e, Digital Equipment Corporation).

**Results**

**Effects of Stimulation Frequency and External Calcium Concentration on Steady State Duration**

The effects of rapid stimulation and elevated calcium concentration on the Purkinje fiber action potential are compared in Figure 1. The records were taken from the same cell during consecutive trials in Tyrode’s solutions containing 1.8 and 7.2 mM calcium. Panel A shows the effect of an increase in the rate of stimulation with external calcium held constant at 1.8 mM. Increasing the stimulus frequency from 30 (trace a) to 95 pulses/min (trace b) shortened and steepened the plateau, without greatly altering the time course of the final phase of repolarization (phase 3). Panel B shows the effect of raising calcium concentration to 7.2 mM. The 4-fold increase in extracellular calcium concentration at a constant stimulation frequency of 30 pulses/min shortened the action potential and modified its shape (trace c; trace a is the same record shown in panel A). The plateau became more rounded, without much change in height, and terminal repolarization was prolonged and more conspicuous. The effects of elevated calcium were reversible and are consistent with those reported previously for Purkinje fibers (Temte and Davis, 1967).

The frequency dependence of the action potential in 7.2 mM calcium is illustrated in panel C.
Increasing the stimulus frequency from 30 (trace c) to 95 pulses/min (trace d) further shortened the action potential and increased the slope of the plateau. Phase 3 was relatively unaltered. These changes resemble those in normal Tyrode's solution following the same step in frequency (cf., panel A), except that the plateau height appears more frequency sensitive when the external calcium level is high.

A quantitative summary of nine such experiments is presented graphically in Figure 2. The solid lines fitted to the filled symbols show the steady state frequency-duration relation for cells in normal Tyrode's solution containing 1.8 mM calcium. Action potential duration decreased linearly with increasing frequency between 30 and 180 pulses/min. No significant differences existed between the slopes of the lines representing the three levels of repolarization, underscoring the earlier observation that rapid stimulation shortens the action potential primarily through changes in the duration of the plateau phase. The dashed lines fitted to the open symbols indicate the effect of elevated extracellular calcium on the frequency-duration relation for the same population of cells. Note that 7.2 mM calcium decreased the action potential duration over the entire range of frequencies studied, and that these changes were nearly uniform for each level of repolarization used to measure duration. The linear dependence of action potential duration on stimulus frequency was retained in high calcium, although the slope of the relationship became less steep compared to control. The decrease in slope, however, was not statistically significant.

Voltage clamp experiments have suggested that the duration of the cardiac action potential depends most directly on the length of the preceding diastolic period, rather than the time between successive stimuli (Hauswirth et al., 1972). Since factors such as elevated calcium, which shorten the action potential at fixed cycle length, reciprocally lengthen the diastolic interval, it is perhaps more appropriate to relate steady state duration to diastolic interval rather than stimulation frequency per se, i.e., total cycle length. In Figure 3, the data from the previous figure have been replotted against diastolic interval, defined as the time for which the membrane potential remains negative to −60 mV (see Methods). The data presented were measured at −60 mV in

![Figure 2](image-url)  
**Figure 2** Effect of external calcium concentration on the steady state frequency-duration relation. The data represent mean steady state (standard) durations (n = 9) measured at −25 (△, ■), −60 mV (○, ●), and 95% of maximum diastolic potential (▲, ▲) during consecutive trials in normal Tyrode's, containing 1.8 mM calcium (filled symbols), and in calcium-rich Tyrode's, containing 7.2 mM calcium (unfilled symbols). Standard error bars were omitted for clarity; standard errors were less than 2% of the measured durations. Linear regression analysis of these data yielded straight line fits with statistically identical slopes and correlation coefficients all greater than 0.983.

![Figure 3](image-url)  
**Figure 3** Steady state action potential duration (standard duration) as a function of diastolic interval. A: The data are mean standard durations (n = 9) measured at −60 mV in 1.8 (●) and 7.2 (○) mM calcium, replotted from Figure 2. B: The same data as in panel A after normalization to the standard duration at 30 pulses/min for each solution.
1.8 (filled symbols) and 7.2 mM calcium (unfilled symbols), but equivalent curves can be constructed using measurements of duration at ~25 mV or 95% of total repolarization. Steady state action potential duration is a smooth function of diastolic interval and appears to show the same interval dependence in both normal and calcium-rich solutions. This is seen more clearly in panel B, where steady state duration at each test frequency is expressed as a percent of the steady state duration measured at 30 pulses/min. Normalized in this way, all points, regardless of the external calcium concentration, fall on a single curve describing a common interval dependence.

**Effects of Stimulation Frequency and External Calcium Concentration on the Interval-Duration Relation**

The relationship between the duration of an extrasystolic response and the immediately preceding diastolic interval observed under control conditions (1.8 mM Ca\(^{2+}\)) is shown in Figure 4. The steady state stimulation frequency of 6 pulses/min was selected to establish the shape of the relation under conditions permitting nearly full recovery of the repolarization currents (Hauswirth et al., 1972; McAllister et al., 1975). Maximum diastolic potential in this particular cell was ~94.7 mV. It is evident that the test duration was markedly affected by changes in diastolic interval (Fig. 4, panel A). Relatively little shortening of the test response was seen for intervals greater than about 1 second. At diastolic periods shorter than this, however, very large changes in test duration occurred. Panel B shows that the relationship can be resolved into two exponential phases. In this example, the time constant of the initial rapid phase was 152 msec and that for the slower later phase was 1042 msec. In six individual control experiments in 1.8 mM calcium, the fast time constant averaged 115 ± 13 msec (mean ± SEM), and the slow time constant averaged 934 ± 53 msec (maximum diastolic potential, 92.1 ± 1.3 mV). The initial rapid change correlates well with the time course of deactivation of \(i_x\) at diastolic potential measured using voltage clamp (Noble and Tsien, 1969a; 1969b; Hauswirth et al., 1972; McAllister et al., 1975). The interpretation of the slower component is more complicated since a number of separate currents may contribute to the changes (Noble and Tsien, 1972). For example, changes in the positive dynamic current (Peper and Trautwein, 1968), the slow inward current (Gibbons and Fozzard, 1975), and the pacemaker current, \(i_K\) (Noble and Tsien, 1968), all of which have time constants of about 1 second at diastolic potential, may participate in this process. The experiments reported herein were not designed to distinguish among these possibilities and, therefore, no further data on the slow components are presented.

The influence of stimulation frequency on the interval-duration relation is shown in Figure 5. In this experiment, premature action potentials were initiated during diastole at steady state frequencies of 6, 24, 60, and 96 pulses/min. As shown in panel A, the major effect of increased steady state frequency was a progressive downward displacement of the curve. The unfilled symbol at the right of each curve is the steady state duration for the basic stimulation frequency of 6 pulses/min. Normalized in this way, all points, regardless of the external calcium concentration, fall on a single curve describing a common interval dependence. This is seen more clearly in panel B, where steady state duration at each test frequency is expressed as a percent of the steady state duration measured at 30 pulses/min. Normalized in this way, all points, regardless of the external calcium concentration, fall on a single curve describing a common interval dependence.
stimulation frequency. The superimposition of such normalized data was observed in 18 of 18 experiments. In view of this agreement, subsequent interval-duration analyses used exclusively background steady state frequencies of 24, 30, and 60 pulses/min. These frequencies allowed ample time for evoking an adequate number of test responses from a stable diastolic potential.

Also shown in Figure 5B (unfilled symbols), plotted as a function of diastolic interval, are the steady state durations for frequencies of 24–120 pulses/min expressed arbitrarily as percents of the standard duration at 6 pulses/min. The difference between the curve connecting these points (dashed line) and the normalized interval-duration curve (solid line) represents the additional amount of shortening undergone by the action potential during the transition from nonsteady to steady state. Similar results were obtained in all 18 experiments of this kind.

A 4-fold elevation of the extracellular calcium concentration had effects on the interval-duration relation resembling those just described for increases in stimulation frequency. Raising the extracellular calcium level from 1.8 to 7.2 mM typically reduced the steady state duration of the action potential and shifted the interval-duration curve downward to correspondingly shorter durations. Figure 6, A and B, shows this result for premature action potentials recorded in normal and calcium-rich Tyrode’s solution at basic stimulation frequencies of 30 and 60 pulses/min. Figure 7 shows the same data...
normalized to the duration of the immediately preceding steady state action potential under each set of conditions. Comparison of recovery time constants from nine such experiments showed that elevated calcium had no significant effect \( (P < 0.5) \) on the interval-duration relation. The interval dependence of the action potential is clearly not influenced by the level of calcium in the bathing solution, both during premature excitation, and in the steady state (cf., Fig. 3).

**Effects of Calcium Channel-Blocking Agents on Steady and Nonsteady State Action Potential Duration Relations**

Steady state frequency-duration and interval-duration analyses, as described above, were performed in the presence of the calcium channel-blocking agents, manganese and verapamil. The concentrations used (3.6 mM manganese and 0.5–6.0 mg/liter of verapamil) have been reported to block slow inward current in voltage clamp experiments (Kohlhardt et al., 1972, 1973) and to abolish calcium-dependent slow activity in dog Purkinje fibers (Cranefield et al., 1974). Addition of either agent to normal Tyrode’s solution resulted in an almost immediate cessation of visible mechanical activity. This was associated with a depression of the plateau phase and the extension of terminal repolarization as reported previously (Cranefield et al., 1974; Rosen et al., 1974; Hogan and Spitzer, 1975; Kass and Tsien, 1975). Examples of such changes are shown in Figure 8 for a fiber exposed to 3.6 mM manganese. The changes in action potential waveform were stable within 15 minutes after beginning superfusion and were readily reversible upon return to control Tyrode’s solution. Figure 8 shows the superimposed response of the same cell during control (solid tracing) and in the presence of manganese (dashed tracing) during steady state stimulation at 60, 120, and 180 pulses/min. Note that, despite the marked effects of manganese on the time course of repolarization, the response to decreasing diastolic interval was the same uniform shortening seen under control conditions. The persistence of uniform action potential shortening in the presence of manganese and verapamil justified the application of the same definitions and procedures used earlier to quantify changes in duration in normal and calcium-rich solutions.

The effects of 3.6 mM manganese on the steady state frequency-duration relation are shown in Figure 9. The data presented in panel A are the mean standard durations measured at −25 mV ( ), −60 mV ( ), and 95% of the maximum diastolic potential ( ) during consecutive runs in control Tyrode’s solution (filled symbols) and after addition of 3.6 mM manganese to the bathing solution (unfilled symbols). These data are plotted as a function of frequency and have been fitted by straight lines having statistically identical slopes with correlation coefficients greater than 0.97. Standard error bars have been omitted for clarity; standard errors averaged less than 4% of the measured duration. B: The standard duration data measured at −60 mV from panel A replotted as a function of diastolic interval. Each data point has been normalized to the standard duration at 24 pulses/min in control solutions ( ) and in manganese-containing solution ( ).

**Figure 8** Changes in action potential time course produced by 3.6 mM manganese at steady state frequencies of 60, 120, and 180 pulses/min. The solid tracings were taken in normal Tyrode’s solution and the broken tracings in Tyrode’s containing 3.6 mM manganese. Upstrokes have been lined up to facilitate comparison of the repolarization process.

**Figure 9** The effect of manganese on the steady state frequency-duration relation. A: Data represent mean standard durations measured at −25 mV ( ), −60 mV ( ), and 95% of the maximum diastolic potential ( ) during consecutive runs in control Tyrode’s solution (filled symbols) and after addition of 3.6 mM manganese to the bathing solution (unfilled symbols). These data are plotted as a function of frequency and have been fitted by straight lines having statistically identical slopes with correlation coefficients greater than 0.97. Standard error bars have been omitted for clarity; standard errors averaged less than 4% of the measured duration. B: The standard duration data measured at −60 mV from panel A replotted as a function of diastolic interval. Each data point has been normalized to the standard duration at 24 pulses/min in control solutions ( ) and in manganese-containing solution ( ).
phases of repolarization, as shown in Figure 8. At 
-25 mV, the duration of the action potential is 
decreased by roughly 40 msec, whereas at 95% of 
total repolarization, it is lengthened by a similar 
amount. Little change is evident at -60 mV. The 
parallel nature of the relations indicates that the 
frequency-dependent abbreviation of the action po-
tential in manganese occurs largely through 
changes in the duration of the plateau phase, as is 
the case in both normal and calcium-rich situations.

In eight additional experiments, the application of 
verapamil, 1.0 mg/liter, had the same general effect 
on the steady state frequency-duration relation as 
did manganese. At each of the three levels of re-
poliarization measured, the relations were linear with 
no significant differences between control and 
verapamil-treated cells (P < 0.5). As in the presence 
of manganese, the duration at -25 mV was uni-
formly depressed at rates between 30 and 180 
pulses/min by about 32 ± 3 msec and at 95% of 
total repolarization lengthened uniformly by 25 ± 
7 msec.

In panel B, the frequency-duration relations mea-
sured at -60 mV in control and manganese-con-
taining Tyrode's solution have been replotted as a 
function of diastolic interval. The data have been 
normalized in each case to the durations obtained 
in each solution at frequency of 24 pulses/min. It is 
apparent that the data described the same curve, 
suggesting that the interval dependence of action 
potential duration in the steady state is unaltered 
by manganese.

The effects of manganese and verapamil on the 
interval-duration relation were tested in a total of 
10 different preparations. The results from two 
experiments are shown in Figure 10. Panel A com-
pares interval-duration relations recorded in control 
Tyrode's (filled circles) and in Tyrode's containing 
3.6 mM manganese (unfilled circles). The protocol 
followed in these experiments is the same as de-
scribed earlier. Extrasystoles were elicited during 
the early part of diastole against a basic stimulation 
frequency of 30 pulses/min, and the duration of the 
premature response (test duration) was plotted as 
a function of the preceding diastolic interval. The 
test durations have been normalized to the duration 
of the steady state (standard) action potential re-
covered at —60 mV in control and manganese-con-
taining Tyrode's solution. It is evident that the data 
shown in Figure 8 represent the same curve, 
suggesting that the interval dependence of action 
potential duration in the steady state is unaltered 
by manganese.

In panel B, the frequency-duration relations mea-
sured at -60 mV in control and manganese-con-
taining Tyrode's solution have been replotted as a 
function of diastolic interval. The data have been 
normalized in each case to the durations obtained 
in each solution at frequency of 24 pulses/min. It is 
apparent that the data described the same curve, 
suggesting that the interval dependence of action 
potential duration in the steady state is unaltered 
by manganese.

FIGURE 10 Changes in the interval-duration relation in response to calcium antagonists. A: Test durations were plotted against the length of the preceding diastolic interval during consecutive runs in control Tyrode's (●) and Tyrode's containing 3.6 mM manganese (○) as a function of basic stimulation frequency of 30 pulses/min. The data appear as percents of steady state duration obtained under each condition. B: Interval-duration relations obtained in control Tyrode's (○) and after the addition of verapamil in concentrations of 1 mg/liter (○) and 3 mg/liter (●). Time constants calculated for each curve are shown in the lower left corner of each panel.

was raised further to 3.0 mg/liter (unfilled squares). 
The time constants for the relation increased pro-
gressively from 122.4 msec in control to 172.0 and 
271.4 msec in verapamil 1.0 and 3.0 mg/liter, re-
spectively. In six experiments, 1.0 mg/liter of 
verapamil increased the time constant by 34 ± 8 
msec, a significant increase over control (P < 0.01). 
The changes in time constant cannot be attributed 
to variations in the maximum diastolic potential, 
since this parameter remained fairly constant (±2 
mV) throughout these experiments.

Discussion

In the present study, action potential duration was 
found to vary directly with diastolic interval, 
both in the steady state and during premature 
excitation, a finding that is in substantial agreement 
with previous reports (for review, see Carmeliet, 
1977). The changes in duration seen with both 
patterns of stimulation are believed to be related to
The action of manganese and verapamil on the stimulation typically were longer than 200 msec. In the present study, diastolic intervals during steady state action potentials recorded in the presence of time-independent outward current in ventricular muscle after loading the cells with calcium by increasing the frequency of voltage clamp steps or by inhibiting the sodium-calcium exchange (Reuter and Seitz, 1968) by removal of external sodium. A role for calcium entry via slow inward current was suggested by the observation that D600 and verapamil inhibited the increase in outward current produced by rapid stimulation.

In our study, blockade of the slow inward channel by manganese and verapamil did not prevent the frequency-dependent changes in duration and shape of the Purkinje fiber action potential. Thus, if calcium entry during rapid stimulation does modulate action potential duration, as has been proposed (Bassingthwaighte et al., 1976), then alternate pathways for Ca\(^{2+}\) exchange also must be important (e.g., sodium-calcium exchange). Katzung et al. (1973) found that the slow inward current channel and the sodium-calcium exchange were separate processes which would be differentially inhibited by lanthanum. The argument for calcium entry via alternate pathways is further supported by the work of Kass and Tsien (1976), which demonstrated that a calcium-mediated increase in the background potassium current could be dissociated from the activation of slow inward current.

On the basis of these results, we conclude that the mechanisms underlying the sudden changes in action potential duration with premature excitation and the more gradual changes seen with repetitive stimulation are separate processes which can be dissociated temporally and pharmacologically. The data suggest, but do not prove, that intracellular calcium may modulate some of these changes. We are at this time unable to exclude completely other hypotheses explaining action potential shortening during repetitive stimulation, such as electrogenic sodium pumping (Gadaby and Cranefield, 1979) or potassium accumulation outside the cell (Hoffman and Cranefield, 1960; Kline and Morad, 1976). It will be of considerable interest to determine the basis for these changes more fully.

Acknowledgments
We wish to acknowledge the excellent assistance of Phyllis Parisi, Mary Ellen Friedman, and Mary Lou Taggart in the preparation of this manuscript.

References
Bassingthwaighte JB, Fry CH, Mcguigan JAS (1976) Relationship between internal calcium and outward current in mammalian ventricular muscle; a mechanism for the control of the action potential duration? J Physiol (Lond) 262: 15-37
Beeler GW, Reuter H (1977) Reconstruction of the action potential of ventricular myocardial fibres. J Physiol (Lond) 268: 177-210
Colatsky TJ, Hogan PM (1976) Evidence that calcium and frequency shorten the action potential through increases in net outward background current (abstr). Physiologist 19: 154
Gadsby DC, Craneffield PF (1979) Electrogenic sodium extrusion in cardiac Purkinje fibers. J Gen Physiol 73: 819-837
Gibbons WR, Fozzard HA (1975) Slow inward current and contraction of sheep cardiac Purkinje fibers. J Gen Physiol 65: 367-384
Isenberg G (1975) Is potassium conductance of cardiac Purkinje fibers controlled by \( [Ca^{2+}] \)? Nature 253: 273-274
Isenberg G (1977) Cardiac Purkinje fibers: Resting, action, and pacemaker potential under the influence of \( [Ca^{2+}] \), as modified by intracardial injection techniques. Pfluegers Arch 371: 51-59
Katzung BG, Reuter H, Porzig H (1973) Lanthanum inhibits Ca inward current but not Na-Ca exchange in cardiac muscle. Experient 29: 1073-1075
Noble D, Tsien RW (1968a) The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. J Physiol (Lond) 195: 185-214
Noble D, Tsien RW (1969b) Reconstruction of the repolarization process in cardiac Purkinje fibres based on voltage clamp measurements of membrane current. J Physiol (Lond) 200: 153-161
Effects of external calcium, calcium channel-blocking agents, and stimulation frequency on cycle length-dependent changes in canine cardiac action potential duration.

T J Colatsky and P M Hogan

Circ Res. 1980;46:543-552
doi: 10.1161/01.RES.46.4.543

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/46/4/543.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/