Possible Mechanisms of Ventricular Arrhythmias Elicited by Ischemia followed by Reperfusion

Studies on Isolated Canine Ventricular Tissues

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SUMMARY. The purpose of this study was to develop an isolated tissue model in which arrhythmic activity could be generated in response to conditions encountered in ischemia followed by reperfusion, and in which intracellular recordings could be used to identify and study arrhythmogenic mechanisms. Isolated canine Purkinje fiber-papillary muscle preparations were superfused with modified Tyrode's solutions. Tissues were exposed to conditions observed in ischemia (hypoxia, acidosis, elevated lactate, zero substrate for 40 minutes). Superfusion with Tyrode's solution of "normal" composition was then reinstituted. Transmembrane recordings from Purkinje and muscle tissues were made, using standard microelectrode techniques. Ischemic conditions caused loss of membrane potential, shortened action potentials, depressed excitability, and progressive bidirectional conduction block between muscle and Purkinje tissues. Spontaneous activity, probably reentrant in origin, was observed. Return to nonischemic conditions resulted in a multiphasic sequence of responses in Purkinje fibers: prompt hyperpolarization, progressive depolarization to unresponsiveness, and final repolarization to control. The depolarization phase was accompanied by oscillatory afterpotentials which initiated extrasystoles. Final repolarization included a phase of automaticity at low membrane potentials, during which Purkinje tissue functioned as a parasystolic focus. Elevation of potassium concentration to 10 mM during the ischemic period did not alter the sequence of electrophysiological events during ischemic conditions or upon reperfusion. This study demonstrates that ischemia followed by reperfusion elicits an orderly sequence of electrophysiological events which may constitute important mechanisms of arrhythmia in vivo. (Circ Res 56:184-194, 1985)

CORONARY ligation in the dog leads to arrhythmias with early and late phases (Tennant and Wiggers, 1935; Harris and Rojas, 1943; Harris, 1950). However, reperfusion of previously ischemic myocardium also leads to a rapid increase in arrhythmias including ventricular fibrillation (Harris and Rojas, 1943; Harris, 1950). Mechanisms of arrhythmias during the two periods may differ (Corbalan, 1976). Arrhythmias during the early phase following coronary ligation may be caused by reentry (Lazzara et al., 1978). However, injury currents at the junction of ischemic and normal tissues also may enhance automaticity during this phase (Janse et al., 1980). Arrhythmias in response to reperfusion may involve enhanced automaticity, possibly in addition to reentry (Penkoske et al., 1978; Murdock et al., 1980; Kaplinsky et al., 1981). Possible mechanisms of automaticity include phase 4 depolarization characteristic of ventricular Purkinje fibers, or abnormal mechanisms such as oscillatory afterpotentials (delayed afterdepolarizations) or depolarization-induced automaticity (early afterdepolarizations).

The purpose of this study was to develop an in vitro model in which arrhythmic activity could be generated in response to ischemia and reperfusion, and in which intracellular recordings could be utilized to differentiate between the mechanisms. We studied the endocardial aspect of ventricular preparations because both muscle and Purkinje tissues are accessible and because all, except the most superficial layers of large bundles of Purkinje fibers, may be subjected to adverse conditions in response to ischemic episodes in vivo (Lazzara et al., 1974). Also, we wished to study effects of ischemia and reperfusion on conduction across the Purkinje-myocardial junction. A preliminary report of this work has been published in abstract form (Ferrier, 1983).

Methods

Experiments were performed on cardiac tissues excised from adult mongrel dogs (15-20 kg) of either sex. Hearts were rapidly removed from animals anesthetized with sodium pentobarbital (30-35 mg/kg, iv). The hearts were fibrillated electrically, and papillary muscles with attached branches of the specialized conducting system (false tendons) were excised from either ventricle and mounted in a tissue bath for study. The tissues were superfused with a modified Tyrode's solution which was equilibrated with 95% O_2/5% CO_2 gas mixture and maintained at 37°C. Stimulation was accomplished through bipolar silver electrodes which were applied either to the false tendon or to the apex of the papillary muscle. Stimuli were rectangular pulses, 3 msec in duration, and were 1.5 times
the threshold voltage. Pulses were obtained by an optically isolated digital stimulator (Pulsar 41 and 61, Frederick Haer and Co.). Preparations were stimulated with patterns of stimulation consisting of trains of 10 or 15 pulses followed by 3-second pauses. The basic cycle length (BCL) was usually 500 msec, but could be varied, so that we might study effects on subsequent activity in the pause.

Transmembrane activity was recorded at two sites, using glass microelectrodes filled with 2.7 M KCl (resistance 15–30 MΩ). One microelectrode was impaled in the free-running segment of a false tendon, the other was usually impaled in a muscle fiber in the apical one-third of the papillary muscle. The microelectrode recordings and record of stimulus pattern were displayed on an oscilloscope (Tektronix 5110) and photographed with a Grass camera.

The experimental protocol included an initial equilibration period of 1–2 hours, during which the tissues were superfused with control Tyrode’s solution with the following millimolar composition: NaCl, 129.0; KC1, 4.0; NaH2PO4, 0.9; NaHCO3, 20.0; CaCl2, 2.5; MgSO4, 0.5; and dextrose, 5.5. The pH was 7.4 when the solution was gassed with 95% O2/5% CO2 and warmed to 37°C. After equilibration, control activity was recorded. The preparations then were superfused for 40 minutes with Tyrode’s solution with a composition which had been further modified to mimic several of the conditions occurring in ischemia (Elharrar and Zipes, 1977). The millimolar composition of the “ischemic” Tyrode’s solution was: NaCl, 123.0; KCl, 4.0; NaH2PO4, 0.9; NaHCO3, 6.0; CaCl2, 2.5; MgSO4, 0.5; and sodium lactate, 20.0. This solution was gassed with 95% N2/5% CO2, resulting in a final Po2 of 48 mm Hg measured in the tissue bath. The pH of the solution at 37°C was 6.8. Thus, the test solution mimicked hypoxia, acidosis, lactate accumulation, and substrate deprivation. In one series of six experiments, the effects of elevating the concentration of potassium during the “ischemic” period were studied. In those experiments, the concentration of KCl was 10 mM. This potassium concentration was selected to correspond to the mean plateau level reported by Hill and Gettes (1980) for extracellular myocardial potassium levels during coronary occlusion. In all experiments, the preparations were exposed to test solution for 40 minutes. Superfusion with control Tyrode’s solution was then reinstated, and the effects were monitored for 1 hour. Control and test solutions were delivered to the tissue chamber via separate conduits at a flow rate of 10 ml/min. The bath volume was 10 ml. Thus, transition from one solution to another was rapid, although not instantaneous.

Results

The effects of exposing false tendon-papillary muscle preparations to conditions of ischemia followed by reperfusion proved to be complex but reproducible. Description of these effects is facilitated by considering periods of ischemia and reperfusion separately.

The effects of ischemic conditions in two representative experiments are illustrated in Figure 1. Panels A and B were recorded before exposure to ischemic conditions in two representative experiments are illustrated in Figure 1. Panels A and B were recorded before exposure to ischemia. Panel C was recorded during the control, nonischemic period. Panel D was recorded after exposure to ischemic conditions for 35 minutes. Panel E was recorded during the control, nonischemic period. Panel F was recorded after exposure to ischemic conditions for 10 minutes. Time and voltage calibrations, and zero reference potentials are indicated in panel F. In panels E and F, MDP = maximum diastolic potential; CT = conduction time. The time calibration for panels A–D (shown in panel D) is 2 seconds for panels A and C, and 100 msec for panels B and D. The voltage calibration for panels A–D is given beside panel C, and zero reference potentials for microelectrode recordings are given in panel A. Panels E and F, from another preparation, show transient enhancement of pacemaker activity in Purkinje tissue by conditions of ischemia. Panel E was recorded during the control period. Panel F was recorded after a 10-minute exposure to ischemic conditions. Time and voltage calibrations, and zero reference potentials are indicated in panel F. In panels E and F, MDP = maximum diastolic potential in Purkinje tissue.

FIGURE 1. Effects of ischemic conditions on the electrophysiological characteristics of canine Purkinje and muscle tissues. In each panel, the top trace is a microelectrode recording from false tendon (Purkinje tissue), the second trace is a recording from papillary muscle, and the bottom trace is a record of stimulation delivered to the false tendon. Panels A and B were recorded during the control, nonischemic period. Panels C and D were recorded after exposure to ischemic conditions for 35 minutes. MDP = maximum diastolic potential; CT = conduction time. The time calibration for panels A–D (shown in panel D) equals 2 seconds for panels A and C, and 100 msec for panels B and D. The voltage calibration for panels A–D is given beside panel C, and zero reference potentials for microelectrode recordings are given in panel A. Panels E and F, from another preparation, show transient enhancement of pacemaker activity in Purkinje tissue by conditions of ischemia. Panel E was recorded during the control period. Panel F was recorded after a 10-minute exposure to ischemic conditions. Time and voltage calibrations, and zero reference potentials are indicated in panel F. In panels E and F, MDP = maximum diastolic potential in Purkinje tissue.
ischemic conditions. In Purkinje tissue (panel A, top record), a train of 15 driven responses was followed by slow diastolic depolarization which failed to initiate activity within a 3-second pause in stimulation. Panel B illustrates the configurations of the potentials recorded from Purkinje and muscle tissues. The maximum diastolic potentials (MDP) recorded in Purkinje and muscle tissues respectively were —90 and —82 mV. Panels C and D were recorded after exposure to ischemic conditions for 35 minutes. In nine preparations in which stable impalements were maintained the MDP in Purkinje decreased 12.5 ± 3.9 mV (SE). Stable impalements were maintained in muscle in six experiments. Here, the decrease in MDP was 9.6 ± 4.3 mV (SE). The decrease in MDP was accompanied by changes in action potential configuration (panel D). In Purkinje fibers, ischemia caused the APD at 25, 50, and 95% of repolarization to decrease by 55.4 ± 10.7 (SE), 57.0 ± 10.3, and 14.0 ± 10.6 msec, respectively. In muscle, the corresponding abbreviations were 21.0 ± 7.7 (SE), 21.1 ± 10.3, and 4.9 ± 4.7 msec. Thus, in both tissues, the time to complete repolarization was not greatly altered. However, the plateau was markedly depressed, and the action potential duration early in phase 3 repolarization was abbreviated.

In two of nine experiments, automaticity was transiently enhanced by exposure to ischemic conditions for approximately 10 minutes. Recordings from one experiment are shown in Figure 1 (E and F). Before exposure to ischemic conditions, driven activity in Purkinje tissue was followed by phase 4 depolarization which failed to reach threshold (panel E). Panel F was recorded from the same fiber after exposure to ischemic conditions for 10 minutes. The slope of phase 4 depolarization was greatly enhanced and, during the pause in stimulation, three spontaneous beats were generated and propagated to muscle. After 90 seconds, automaticity stopped (not illustrated), and the slope of phase 4 depolarization gradually declined with further exposure to ischemic conditions. Automaticity was absent in all experiments at the end of the "ischemic period."

Ischemic conditions consistently depressed conduction between Purkinje and muscle tissues. Figure 1 shows an example of first-degree block. Conduction time from the recording site on the false tendon to the site on muscle (determined from enlarged
projections of film records) increased from 16 msec during the control period (panel B) to 22 msec when the preparation was exposed to ischemic conditions (panel D).

Conduction block frequently progressed to second-degree block resembling either Mobitz type I or II patterns. Figure 2 (A–C), recorded after exposure to ischemic conditions for 12 minutes, shows an example of type I or Wenckebach periodicity. Panel A, recorded at a slow sweep speed, demonstrates one-to-one conduction from Purkinje tissue (top trace) to muscle when the BCL was 500 msec. Panel B shows only the upstrokes of the action potentials recorded under the same conditions. In panel B, the earliest upstroke recorded from muscle (lower) corresponds to the first beat of the train. The conduction time from Purkinje tissue to muscle increased progressively with each sequential beat. When the BCL was shortened to 300 msec (panel C), conduction time increased with the first 3 to 4 beats. Conduction then failed for the remainder of the train. Conduction resumed with the beginning of each new train (not illustrated).

Panels D, E, and F of Figure 2 were recorded from another preparation, after exposure to ischemic conditions for 8, 28, and 40 min, respectively. Panel D was recorded before any major depression of conduction and all beats propagated from Purkinje tissue D (top) to muscle (bottom). Panel E was recorded after the conduction delay between Purkinje and muscle sites began to increase progressively through each train (as in panels A and B). Midway through the train, a bigeminal rhythm developed at the recording site in Purkinje tissue. The fixed coupling and degree of prematurity of the spontaneous activity suggests, but does not prove, that the rhythm was reentrant in origin. The abrupt cessation of the arrhythmia with termination of pacing also is compatible with this interpretation. With further exposure of the tissues to the test solution, a higher degree of block appeared and the bigeminal rhythm stopped (panel F). The pattern of second-degree block in panel F is that of Mobitz type II. In the complete series of experiments, depression of conduction was accompanied by nondriven activity ranging from isolated extrasystoles to salvos and short runs of potentials with brief intervals.

Effects of Reperfusion with Normal Tyrode’s Solution

Several distinct phases of arrhythmic activity occurred in sequence when preparations were reexposed to control, oxygenated Tyrode’s solution. The successive appearance of these phases was closely related to a reproducible cyclic change in MDP. Figure 3 shows a typical example of the changes in MDP that occurred in Purkinje tissue during ischemic conditions and the return to nonischemic conditions. Ischemic conditions resulted in prompt decrease in MDP, followed by a period (approximately 30 minutes), during which the MDP remained relatively stable. Near the end of the 40-minute exposure to ischemic conditions, the MDP decreased further. Return to control Tyrode’s solution resulted in a rapid repolarization which, in two experiments, resulted in hyperpolarization of the Purkinje tissues relative to the control MDP. Maximum repolarization of the tissues was achieved within 2–4 minutes of reperfusion, and was followed by rapid depolarization to membrane potentials of −55 to −60 mV. The false tendons remained severely depolarized or recovered very slowly for 15–30 minutes. Following this period, recovery progressed more rapidly. Stable membrane potentials −80 mV or greater were achieved within 50–60 minutes of reperfusion with control Tyrode’s solution. Essentially identical phasic changes in membrane potential were seen in all nine preparations in which stable impalements were maintained throughout the experiment. Impalements in muscle were seldom maintained throughout the period of reperfusion. The discontinuous recordings which were collected indicate that muscle exhibited qualitatively similar, but greatly attenuated, phasic changes in MDP.

Several different mechanisms or potential mechanisms of arrhythmia appeared in response to return to nonischemic conditions. The sequence of their appearance and the relationship to the changes in MDP are illustrated in Figure 4. Except where noted, the top microelectrode recording is from the Purkinje site and the bottom record is from muscle. The MDP recorded in Purkinje tissue is given for each panel. Panel A, identical to panel F of Figure 2, was recorded at the end of the period of ischemic conditions. The MDP of Purkinje tissue had decreased to
−68 mV and second-degree conduction block had developed. In panel B, recorded 3.5 minutes after return to control Tyrode’s solution, second-degree conduction block was still evident. However, the MDP in Purkinje tissue had increased to −83 mV. The MDP in muscle had also increased slightly. Immediately after the traces in panel B were recorded, the Purkinje fibers began to depolarize. Panel C was recorded 7.5 minutes after return to control Tyrode’s solution. The MDP had decreased to −64 mV. During this period of progressive depolarization, oscillatory afterpotentials (OAP) appeared in Purkinje tissue but not in muscle. An OAP can be seen following the last driven action potential in the record from Purkinje tissue in panel C. OAP were observed at this phase of progressive depolarization in eight of nine preparations.

The preparation continued to depolarize following the sequence illustrated in panel C. After 8.5 minutes of reperfusion, the MDP had decreased to −58 mV (panel D). The preparation could no longer be driven by stimuli applied to the false tendon. Therefore, stimulation was then delivered to the apex of the papillary muscle. Active responses were still elicited in muscle, but no longer propagated to the recording site on the false tendon. In panel D, the muscle action potentials cross over the trace from Purkinje tissue. The responses in Purkinje tissue are represented by the much slower electrotonic (passive) deflections with amplitudes of approximately 20 mV. Panel E, recorded after 11 minutes of reperfusion, is similar to panel D, except that the rate of stimulation is faster and the microelectrode that was previously impaled in muscle had been reimpaled in a site in subendocardial Purkinje tissues. Activity clearly propagated from muscle to subendocardial Purkinje fibers. Failure of conduction occurred between the subendocardial layer of Purkinje fibers and the false tendon which continued to exhibit only electronic deflections. In five experiments, the second microelectrode was used to impale numerous sites on the false tendon during this stage. All sites were found to be depolarized to levels similar to those seen at the continuously impaled site. These observations plus the finding that the preparations could not be driven by stimuli applied to the false
tendon, indicate that most, if not all, of the fibers within the false tendon were similarly depolarized. Reperfusion with control Tyrode’s solution was followed by depolarization, inexcitability, and failure of conduction in false tendons in all experiments. The membrane potential initially recovered very slowly following this phase. In all experiments, gradual increase in MDP was accompanied by the appearance of pacemaker activity at membrane potentials characterized by “slow response” or calcium-mediated action potentials. This activity has been described in Purkinje tissue (Hauswirth et al., 1969; Imanishi, 1971) and muscle where it has been referred to as depolarization-induced automaticity (DIA) (Katzung and Morgenstem, 1977). An example is shown in panel F of Figure 4, recorded 36 minutes after initiation of reperfusion. The MDP in the Purkinje fiber had increased to −64 mV, and the fiber had become continuously automatic. Stimulation had been discontinued temporarily. There was a smooth transition between the pacemaker potential and the upstroke of the action potential. The lower trace, recorded from a site in muscle, demonstrates that the spontaneous activity originating in Purkinje tissue propagated to the muscle. Automaticity characteristically persisted as the MDP gradually recovered. Panel G was recorded 47 minutes after initiation of reperfusion. The MDP in Purkinje tissue had increased to −78 mV. The train of action potentials starting at the left of this panel represents driven activity initiated by stimuli delivered to a site on muscle. The driven action potentials successfully propagated to the Purkinje site. Pacemaker activity was still exhibited during the pauses in driven activity, but occurred at membrane potentials associated with normal pacemaker activity and sodium-mediated action potential upstrokes. Transition from automaticity at low membrane potentials to the activity shown in panel G was gradual and continuous. Automaticity stopped in all preparations before 60 minutes of reperfusion had elapsed. Panel H was recorded after 50 minutes of reperfusion. The MDP in Purkinje tissue had increased to −81 mV, and automaticity no longer appeared in the pauses in driven activity.

In the present study, reperfusion regularly elicited two mechanisms of abnormal automaticity, OAP and DIA. Although OAP were regularly observed, complete characterization of this abnormal mechanism of automaticity was hindered by the continuously changing response of the tissues. OAP showed a progressive increase in amplitude during the period of depolarization that followed maximum hyperpolarization. This is illustrated in Figure 5A. The panel at the extreme left was recorded at the peak of hyperpolarization following reperfusion with

![Figure 5. Oscillatory afterpotentials induced in Purkinje tissue by return to nonischemic conditions. Panels in 5A illustrate the appearance and progressive increase in amplitude of OAP. The three panels in 5B were recorded from Purkinje tissue in a different experiment from 5A. The basic cycle length (BCL) of stimulation was varied and is indicated in each panel. In the center and right panels, all action potentials were stimulated. The trains of activity were followed by subthreshold OAP. In the left panel, the OAP reached threshold and induced an extrasystole (last action potential). MDP = maximum diastolic potential immediately following the last driven action potential.](http://circres.ahajournals.org//content/83/2/189)
normal Tyrode's solution. This recording from Purkinje tissue shows that the last four driven beats of a train of 15. The MDP at the end of the train was −93 mV. OAP were not visible at this time. The remaining three panels in Figure 5A were recorded from the same impalement and show that the MDP decreased progressively to −85, −75, and −65 mV. OAP appeared following the trains of driven activity, and increased in amplitude as the MDP decreased. The upstrokes of OAP visible in the diastolic intervals between action potentials exhibited a similar increase. The preparation became inexcitable following the sequence illustrated. It is not clear from the present experiments whether the progressive increase in the amplitude of OAP is caused by the progressive decrease in MDP or another mechanism. The amplitude of digitalis-induced OAP increases with depolarization over the same range of membrane potentials (Ferrier, 1980; Wasserstrom and Ferrier, 1981). A similar voltage dependence has been demonstrated for an oscillatory current in sheep Purkinje fiber in the absence of cardiotonic agents (Vassalle and Mugelli, 1981).

The amplitude and interval of OAP induced by digitalis also change in response to the BCL of preceding driven activity (Ferrier et al., 1973). The BCL of driven activity also was varied in the present experiments when OAP were exhibited. Figure 5B shows traces recorded from a Purkinje fiber in a representative experiment. The three panels show progressive depolarization as in Figure 5A. However, the BCL of driven activity was 250, 500, and 700 msec (left to right). When the BCL was 700 msec, the ascending limb of OAP could be seen developing between driven responses throughout the train. A distinct OAP was evident following the last driven response. The interval at which the peak of the OAP followed the upstroke of the last driven response was approximately equal to the preceding BCL. When the BCL was 500 msec, the interval of the OAP was shorter and the amplitude was less. In the experiment illustrated, the OAP reached threshold and initiated an extrasystole when the BCL was 250 msec. In the present experiments, the amplitude of OAP appeared to be least following trains of beats at intermediate BCL (400–500 msec) and increased with either shorter (250–300 msec) or longer BCL (600–700 msec). This relationship appeared qualitatively similar to that documented for digitalis-induced OAP, but could not be quantified in the present study because of the additional effects of continuously decreasing MDP.

Depolarization-induced automaticity also occurred regularly in response to reperfusion with control Tyrode's solution. This abnormal mechanism of automaticity was observed only in Purkinje tissue. The papillary muscles, however, were not markedly depolarized at this time. Previous studies have shown that depolarized cardiac tissues, exhibiting DIA and connected to more normally polarized tissues, may function as parasystolic foci (Ferrier and Rosenthal, 1980; Rosenthal and Ferrier, 1983; Ferrier and Shoukair, 1981). Therefore, the possibilities that false tendons depolarized by ischemic conditions followed by reperfusion would function as parasystolic foci and exhibit electrotonic modulation of spontaneous cycle length were examined in the present study. Figure 6 (A and B) shows traces recorded during reperfusion. The top trace shows that the Purkinje fibers had depolarized and became automatic. The lower microelectrode recording was from muscle and indicates one-to-one exit conduction.

**Figure 6.** Panels A and B: modulated parasystolic activity with exit conduction induced by reperfusion. In each panel, the top trace is a microelectrode record from Purkinje tissue, the middle trace is a microelectrode record from muscle, and the bottom trace is a record of stimuli applied to muscle. Panel C: modulated parasystolic activity with complete exit block. Traces are identified in the text. All three large amplitude rapidly rising action potentials recorded from the surface of the papillary muscle were driven. Voltage and time calibrations are in panel B.
from the focus of automaticity. Of nine preparations examined, six demonstrated exit conduction and three demonstrated third-degree exit block. In Figure 6A, a stimulus was delivered to muscle following the third spontaneous beat. The stimulus elicited an action potential in muscle. The action potential failed to propagate to the recording site in Purkinje tissue. However, it imposed an electrotonic depolarization and caused a marked delay in the next spontaneous firing of the Purkinje fiber. Thus, the false tendon exhibited entrance block to test beats interpolated early in the pacemaker cycle. When test beats were interpolated later with respect to the cycle of the pacemaker, the beats captured the automatic site and, thus, accelerated the discharge of the tissue. An example is shown in panel B. The last three beats in panel B were initiated by stimuli delivered to muscle. The first of these occurred after approximately 40% of the pacemaker cycle had elapsed, and successfully discharged the fiber. The following two driven beats arrived near the expiration of the respective pacemaker cycles and discharged the pacemaker only slightly prematurely. Similar phases of delay and acceleration were demonstrated in seven of nine preparations.

Modulation of pacemaker activity also occurred in preparations exhibiting complete exit block, as shown in Figure 6C. The traces in this example overlap. The trace showing pacemaker potentials graduating smoothly into the upstrokes of action potentials was recorded from the false tendon during reperfusion. The remaining trace was recorded from the surface of the papillary muscle. All three action potentials apparent in the latter trace were elicited by stimuli delivered to the papillary muscle. None of the spontaneous beats generated in the false tendon propagated to the papillary muscle. The driven beats at the middle and right of panel C caused electrotonic depolarizations which delayed the subsequent spontaneous firings of the false tendon. The test beat at the left of panel C occurred late in the spontaneous cycle and accelerated the next firing of the false tendon.

Effects of Elevating Potassium Concentration during Ischemic Conditions

The preceding results were collected in experiments in which the potassium concentrations of the superfusate were 4 mM during both the “ischemic” and reperfusion periods. It is well known that extracellular potassium concentration rises during ischemia (Harris et al., 1954). Our results suggest that elevated potassium is not necessary to elicit a strong reperfusion response in Purkinje tissue. However, elevated levels of extracellular potassium during ischemia might modify or even abolish the reperfusion response elicited in Purkinje tissue. Therefore, in six experiments, we studied the effects of the same sequence of “ischemic” conditions followed by reperfusion, but the potassium concentration was elevated to 10 mM during the “ischemic” period and returned to 4 mM during reperfusion. The high potassium concentration corresponds to the mean plateau level reported for extracellular myocardial potassium levels during coronary occlusion by Hill and Gettes (1980).

The responses of tissues to this modified protocol were similar to those in which potassium was not elevated. The maximum diastolic potential in Purkinje tissue decreased from $-87.8 \pm 1.69$ (SE) mV to $-62.5 \pm 2.6$ (SE) mV when the tissues were exposed to ischemic conditions. The decrease in membrane potential ($25.3 \pm 1.9$ mV) was significantly greater than that observed in response to ischemic conditions at 4 mM KCl ($12.5 \pm 3.9$ mV; $P < 0.02$, unpaired $t$-test). Two preparations exposed to ischemic conditions with 10 mM KCl also exhibited an initial increase in automaticity similar to that seen in experiments using 4 mM KCl (see Fig. 1F). The response of the tissues to reperfusion with normal Tyrode’s solution also was nearly identical to that observed following ischemic conditions with 4 mM KCl. The

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<th>[K+]</th>
<th>Hyperpolarization*</th>
<th>OAP</th>
<th>Depolarization to inexcitability†</th>
<th>DIA</th>
<th>Modulated para-systole</th>
<th>Recovery**</th>
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<td>4 mM</td>
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<td>25.4 ± 6.3‡</td>
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<td>10 mM</td>
<td>Incidence (n = 6)</td>
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<td>Time (min ± SE)</td>
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<td>11.0 ± 8.3†</td>
<td>31.8 ± 5.7‡</td>
<td>42.5 ± 13.1‡</td>
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* Greater than 10 mV relative to end of ischemic period.
† Depolarization to $-50$ to $-60$ mV, inexitable and quiescent.
** Membrane potentials $\geq -80$ mV and cessation of automaticity.
reperfusion responses of tissues exposed to both protocols are summarized in Table 1. The greatest difference observed was that OAP occurred in only two of six of the Purkinje tissues exposed to 10 mM KCl, whereas eight of nine preparations exhibited OAP when the potassium concentration was 4 mM. In addition, although all preparations exhibited a secondary depolarization following the initial hyperpolarization, in one preparation exposed to ischemic conditions with elevated potassium, action potentials initiated by stimulation of muscle continued to propagate into the false tendon throughout the reperfusion period. In the remaining five preparations, depolarization resulted in complete failure of conduction into the false tendon. In these five experiments, DIA also appeared as the preparations began the final recovery phase. Entrance block with modulation of the cycle length of the DIA was demonstrated in four of these preparations (indicated as modulated parasystole in Table 1). All six preparations recovered maximum diastolic potentials greater than -85 mV within 60 minutes of initiation reperfusion. No statistically significant differences in the time courses of the responses were observed with the two concentrations of potassium.

Discussion

The present study demonstrates that canine false tendon-papillary muscle preparations exposed to ischemic conditions followed by return to nonischemic conditions exhibit an orderly sequence of major mechanisms of arrhythmia. As many as five mechanisms may occur in a single ischemia-reperfusion sequence. During exposure to ischemic conditions, Purkinje fibers showed an initial moderate but rapid decline in MDP. This was associated with a transient appearance of automaticity in 25% of preparations. The automatic activity occurred at high membrane potentials (greater than -70 mV) and was therefore presumably generated by normal pacemaker activity characteristic of Purkinje fibers. Continued exposure to ischemic conditions resulted in progressive impairment of conduction across the Purkinje-muscle junction. Spontaneous activity associated with this phase was dependent on an initiating beat for its appearance, and was closely coupled to the initiating beat. The behavior observed is most compatible with a reentrant mechanism, although automatic mechanisms cannot be excluded (Moe et al., 1977). The remaining three mechanisms of arrhythmia occurred in response to return to nonischemic conditions. Reperfusion characteristicly resulted in rapid hyperpolarization followed by depolarization in Purkinje fibers. OAP occurred during the depolarizing phase. OAP were observed when the MDP was between -60 and -85 mV. This range corresponds approximately to the range in which OAP induced by digitalis characteristically appear (Ferrier, 1980; Wasserstrom and Ferrier, 1981). It also is similar to the range of membrane potentials over which an oscillatory current ($I_\omega$) has been reported in sheep Purkinje fibers not treated with digitalis (Vassalle and Mugelli, 1981). Whether the transmembrane current causing OAP upon reperfusion is identical to the current (TI) underlying digitalis-induced OAP (Kass et al., 1978a, 1978b) remains to be determined. The amplitude of the most prominent OAP following a train of driven beats varied with changes in preceding BCL. This relationship also was similar to the cycle length dependence of the amplitude of digitalis-induced OAP (Ferrier et al., 1973). With the conditions of the present study, OAP generated only single extrasystoles. Whether repetitive firing similar to that seen with digitalis-induced OAP (Ferrier et al., 1973) or in 24-hour infarcts (El-Sherif et al., 1983) can be demonstrated with slightly different conditions, remains to be investigated.

The appearance of OAP was followed by progressive depolarization until conduction failed in Purkinje tissue. The MDP of fibers within the false tendon decreased to approximately -55 mV, and the tissue was inexcitable to extracellular stimuli applied directly to the false tendon. In experiments in which one microelectrode was used to impale various sites, the thin subendocardial layer of Purkinje fibers on the surface of the papillary muscle was frequently found to be excitable and less severely depolarized than tissue within the false tendon. Thus, the underlying muscle may provide a protective effect on very nearby Purkinje fibers by acting as a polarizing current source. Failure of conduction also appeared to be restricted to free-running false tendons and thick bundles of Purkinje fibers on the surface of the papillary muscle. This phase of electrical disturbance might be expected to result in macroreentry, or at least disruption of the normal sequence of activation and contraction, if it occurred in the whole heart.

The final recovery of membrane potential in false tendons began with the appearance of automaticity in the depolarized tissue in 14 of a total of 15 preparations. The MDP at this time was approximately -60 mV. Thus the automatic activity was most likely identical to DIA which involves the slow inward (calcium) current as the main inward current (Imanishi, 1971). This mechanism of automaticity exhibits several characteristics which suggest that it may function as an important mechanism of arrhythmia. We have shown that DIA is not subject to overdrive suppression (Ferrier and Rosenthal, 1980). This observation has been confirmed recently (Dangman and Hoffman, 1983). Also, tissues exhibiting DIA, and which are connected to normally polarized tissues, frequently exhibit entrance block and electrotonic modulation of cycle length, as in the present study (Ferrier and Rosenthal, 1980; Ferrier and Shoukair, 1981; Rosenthal and Ferrier, 1983). Modulation and entrainment of the resulting parasystolic focus can generate a wide range of complex arrhythmias, including those with fixed coupling (Jalife and Moe, 1979; Moe et al., 1977).

Experiments which incorporated elevated levels
of potassium as one of the ischemic conditions differed little from those in which potassium was not altered. These observations indicate that elevation of potassium levels is not essential to elicit a potentially arrhythmogenic response during ischemic conditions or upon reperfusion. Downar et al. (1977) also concluded that elevated potassium was neither essential nor sufficient to elicit the electrophysiological changes observed during coronary occlusion of the pig. In the present study, we observed transient enhancement of automaticity during ischemia in one-third of preparations, even in the presence of 10 mM KCl. This may represent strong enhancement of automaticity by lactate and low pH similar to that demonstrated by Coraboeuf et al. (1976). Incorporation of elevated potassium as one of the ischemic conditions in the present study also had little effect on the response during reperfusion. The greatest change we observed was a decreased incidence of OAP. OAP occurred very early in reperfusion, and it is possible that they may have been suppressed in some preparations by slow washout of the higher concentration of potassium.

The responses we have observed to conditions mimicking ischemia followed by reperfusion are not observed in canine false tendon-papillary muscle preparations removed from apparently healthy hearts and exposed only to normal Tyrode's solution. The protocol including initial equilibration, exposure to ischemic conditions, and reexposure to normal Tyrode's solution represents a total duration of 3-4 hours. In various experimental protocols, we have regularly observed preparations exposed to normal Tyrode's solution for periods of up to 10-12 hours. Purkinje tissues which achieved a MDP of -80 mV or greater within 1 hour of dissection were never observed to depolarize spontaneously or exhibit OAP or DIA. Preparations which failed to repolarize following dissection, or exhibited spontaneous beats during control equilibration were excluded from this study. Thus, the observations reported represent the effects of the experimental intervention and are not observed spontaneously.

The conditions incorporated in our model were selected to inhibit energy metabolism in both Purkinje and muscle tissues. Hypoxia was selected to inhibit aerobic metabolism. Substrate exclusion, mimicking near complete substrate extraction in poorly perfused tissue, would aid inhibition of aerobic metabolism, at least in muscle. Purkinje tissue contains large stores of glycogen which could still be utilized anaerobically. Therefore, low pH and elevated lactate were included because they have been shown to inhibit anaerobic metabolism (Rovetto et al., 1975). All of these factors have been demonstrated in ischemic myocardium (Downar et al., 1977; Elharrar and Zipes, 1977). This model is obviously incomplete, since various metabolites (e.g., adenosine, etc.) that would accumulate in the interstitial compartment in a perfusion model must quickly wash out of at least the superficial layers of tissue. On the other hand, the superfusion model has the advantages that one may determine which components are both sufficient and necessary to yield a model that generates recognized mechanisms of arrhythmia, and one can record from both Purkinje and muscle tissues.

In a study of Downar et al. (1977), porcine ventricular tissues exposed to blood from ischemic myocardium in vitro exhibited rapid uneventful recovery upon "reperfusion" with control Tyrode's solution. The reason for absence of marked reperfusion effects could reflect a species difference, differences in exact levels of O2, pH, etc., or perhaps the duration of exposure to ischemic conditions. In their study, isolated preparations were exposed to ischemic conditions for 12 minutes. In preliminary experiments, we have found that 10-minute periods of ischemic conditions are followed by greatly attenuated reperfusion effects.

The main phases of arrhythmogenesis seen in the present study resemble those seen upon occlusion and release of coronary vessels in the in situ canine heart. It is well established that arrhythmias occur during coronary occlusion and that they occur, frequently with greater severity, upon release of the occluded vessel (Harris, 1950; Corbalan et al., 1976; Penkoske et al., 1978; Murdock et al., 1980; Kaplanisky et al., 1981). Arrhythmias occurring early in ischemia are believed to be caused by reentry (Lazzara et al., 1978), and may be subdivided into very early and later periods (Kaplanisky et al., 1979). Arrhythmic mechanisms also occurred in two phases in our model. However, the early phase was related to enhanced automaticity rather than reentry. The significance of this phase is unknown. Late in the ischemic period, activity that appeared to be reentrant was observed.

Our observations are compatible with the occurrence of a major period of arrhythmogenesis upon return to nonischemic conditions. The combination of three different major mechanisms of arrhythmia, OAP, profound conduction block in specialized conducting tissue, and modulated parasystole, may well explain the severity of arrhythmias seen in response to reperfusion following coronary occlusion. Abnormal mechanisms of automaticity may play a major role following reperfusion. Penkoske et al. (1978) studied the effects of 35-minute occlusions of the left anterior descending coronary artery in the cat. Reperfusion was accompanied by improved conduction but marked elevation of idioventricular rate. Enhancement of automaticity may depend on the duration of the ischemic period. Murdock et al. (1980) studied 10-minute occlusions in dogs and did not find an elevation of ventricular rate on reperfusion.

Reperfusion arrhythmias also have been divided by Kaplanisky et al. (1981) into two categories. These authors described an immediate burst of arrhythmic activity during the initial 1-2 minutes. The activity occurred at a time corresponding to the rapid hyperpolarization seen in our in vitro model. If the rapid hyperpolarization occurred at slightly different
times in adjacent tissues, it would be expected to result in marked disparities in excitability and refractoriness and thereby provide conditions favoring arrhythmic activity (Han and Moe, 1964).

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