Spontaneous and Induced Cardiac Arrhythmias in Subendocardial Purkinje Fibers Surviving Extensive Myocardial Infarction in Dogs

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ABSTRACT
The cellular electrophysiological mechanisms underlying the ventricular arrhythmias that accompany myocardial infarction were studied in isolated, superfused infarcted myocardium excised from dogs previously subjected to a two-stage ligation of the anterior descending coronary artery. Ventricular arrhythmias frequently occurred in the intact heart 24 hours after coronary occlusion. Surviving subendocardial Purkinje fibers in infarcts excised at this time were highly arrhythmic when they were studied with intracellular microelectrodes in vitro. These arrhythmias consisted of rapid, repetitive depolarizations and occurred spontaneously or could be induced by premature electrical stimulation. Premature stimulation also resulted in single unstimulated responses. In such instances, premature impulses conducted extremely slowly through the infarcted region where surviving Purkinje fiber action potential durations were extraordinarily prolonged. Conduction block at some sites in the infarct caused phenomena which were interpreted as reentrant beats. Some surviving subependocardial Purkinje fibers in the infarct demonstrated spontaneous diastolic depolarization and appeared to function as pacemakers in the absence of electrical stimulation. In some instances, these fibers constituted typical parasystolic foci, demonstrating both entrance and exit block. These results suggest that subendocardial Purkinje fibers which survive in an infarct may be the site of origin of some of the ventricular arrhythmias that accompany myocardial infarction.

KEY WORDS microelectrode spontaneous diastolic depolarization ventricular tachycardia reentry entrance block parasystole exit block

Despite many studies on the in situ heart after experimental coronary artery occlusion (1-6), the cellular electrophysiological mechanisms underlying the ventricular arrhythmias that accompany myocardial infarction remain unknown. Intracellular recordings of transmembrane action potentials from cells in infarcted regions during these ventricular arrhythmias should yield information about the electrophysiological characteristics responsible for their genesis. Such recordings have been obtained from the epicardium of the in situ heart after coronary artery occlusion (7, 8), but studies of intramural or subendocardial regions of the in situ heart are not readily feasible at present. We have therefore undertaken a different approach to study the alterations in cellular electrophysiology and the mechanisms of ventricular arrhythmias after experimental myocardial infarction. This approach is based on the electrophysiological study of excised, superfused infarcted myocardium with intracellular microelectrodes.

We have previously (9) demonstrated that subendocardial Purkinje fibers survive extensive myocardial infarction. The present study demonstrates that, when arrhythmias are present in the in situ heart, this subendocardial Purkinje network in isolated, superfused infarcted myocardium is arrhythmic in vitro. These results suggest that these fibers may be involved in the genesis of arrhythmias in situ and that this method of study may be useful in the future elucidation of the cellular mechanisms that underlie arrhythmias due to infarction.
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Methods

Experimental myocardial infarction was produced surgically in 23 mongrel dogs (10–14 kg) using the technique previously described by Harris (10). For studies on the arrhythmic heart, 20–24 hours after coronary artery ligation, 7 of these dogs were reanesthetized with sodium pentobarbital (15 mg/kg, iv) and a lead 11 electrocardiogram recorded. At this time ventricular arrhythmias consisting of multifocal ventricular premature depolarizations and periods of ventricular tachycardia interspersed with periods of normal sinus rhythm were present in all dogs. After obtaining the electrocardiogram, the heart was quickly excised through a thoracotomy and placed in a modified Tyrode’s solution bubbled with 95% O2-5% CO2 (9). The infarcted region, along with adjacent noninfarcted tissue, was then dissected free from the remainder of the heart, pinned with the endocardial surface upward to the waxed base of a 50-ml superfusion chamber, and superfused at a rate of 25 ml/min with the Tyrode’s solution. Temperature in the bath was maintained constant at 36.5 ± 0.5°C. Infarcted myocardium appeared pale and was clearly demarcated from the surrounding noninfarcted regions by a distinct border. The dissection procedure and the anatomical structures present in the isolated preparation were identical to those described previously (9). Electrophysiological study was begun within 10 minutes after the excision of the heart. Transmembrane action potentials were recorded from subendocardial fibers in both the infarcted and the control, noninfarcted regions. As described previously (9), action potential generation in the infarcted region was limited to the most superficial one to four cell layers of subendocardial fibers beneath the endocardium. Intracellular recording in the infarcted region was facilitated by the lack of contraction or movement of this area. Although some contraction did occur in the control regions, it did not interfere with the ability to record intracellularly from fibers in these areas. Bipolar electrograms were also recorded from both the control and the infarcted regions with Teflon-coated silver-wire electrodes and a suitable d-c amplifier. Similar studies were also performed on identical preparations obtained from the hearts of 6 normal dogs that had not undergone coronary artery ligation and on preparations obtained from the hearts of 17 dogs excised 3 days to 7 weeks after coronary artery ligation. None of these dogs had ventricular arrhythmias at the time of study.

When possible the isolated preparation was electrically stimulated at a regular rate through bipolar silver-wire electrodes placed on the free-running false tendon which originated from the anterior division of the left bundle branch. Preparations isolated from infarcted hearts 20–24 hours after coronary occlusion demonstrated rapid spontaneous activity for 40–60 minutes after they were mounted in the superfusion chamber (see Results). As a result, during this initial period of study these preparations could not be stimulated consistently at cycle lengths greater than 200 msec. Often even this cycle length was ineffective. After such rapid activity subsided, however, these preparations could be driven at a regular cycle length of 700–800 msec. All other preparations (whether from noninfarcted hearts or from infarcted hearts 3 days to 7 weeks after coronary occlusion) could be stimulated at a cycle length of 800 msec immediately after mounting in the superfusion chamber. Although rapid activity resembling fibrillation was occasionally present in these latter preparations for several seconds to several minutes after mounting, it always ceased immediately on initiation of electrical stimulation.

Premature stimuli, consisting of square-wave pulses 1–3 msec in duration and 1.5–2 times threshold voltage, were delivered to the false tendon either through the same stimulating electrodes used to drive the preparation or to the subendocardial Purkinje fibers through an intracellular microelectrode. In the latter instance, a relay was employed so that the same electrode could be used both for intracellular stimulation and for recording the transmembrane action potential of the stimulated cell. The pulse generators were programed to deliver premature stimuli with variable coupling intervals after every fifth to seventh basic impulse. Premature stimuli were also delivered at random to the subendocardium of spontaneously beating preparations.

In some experiments in which the propagation of premature impulses in the subendocardial Purkinje fiber network of the infarct was studied, two stationary microelectrodes were used to record action potentials from a site near the base of the anterior interventricular septum in noninfarcted tissue and from a site toward the apex of the anterior papillary muscle in the infarcted region. Premature stimuli were delivered through the microelectrode in the noninfarcted tissue. At each coupling interval a third (roving) microelectrode was used to record action potentials from multiple sites. Action potentials recorded at each of these sites were displayed simultaneously with the action potentials recorded from the two fixed sites. By determining the timing of activation of these sites in relation to the two fixed recording electrodes, an attempt was made to determine the pathways of propagation of the premature impulses (Figs. 10, 11).

Results

Rapid, Repetitive Activity in Subendocardial Purkinje Fibers of Infarcted Preparations.—After extensive myocardial infarction resulting from complete occlusion of the left anterior descending coronary artery, subendocardial Purkinje fibers in the infarcted region remain structurally intact and electrophysiologically viable, although all underlying ventricular muscle is necrotic and shows no evidence of electrical activity. In addition, the characteristics of transmembrane action potentials recorded from these fibers are altered (9).

Rapid repetitive generation of action potentials occurred spontaneously in the subendocardial Purkinje fibers of all infarcted preparations isolated from hearts during the arrhythmic stage 20–24 hours after coronary artery occlusion (Fig. 1). (The word
Spontaneous is used in this communication to indicate that action potentials were generated in the absence of electrical stimuli; it does not indicate the mechanism responsible for depolarization. This rapid activity, which was usually evident within minutes after the preparation had been mounted in the perfusion chamber, persisted for 45–60 minutes during superfusion with the oxygenated Tyrode's solution and then gradually subsided. The rapid activity was paroxysmal, appearing in bursts interspersed between periods of quiescence during which electrical stimulation of the false tendon at a regular rate could activate the preparation. Electrical stimulation during the paroxysms of rapid activity usually was ineffective.

During electrical stimulation, rapid activity began with a spontaneous premature depolarization (Fig. 1A). This premature impulse seemed to originate near the border of the infarcted area or in the control, noninfarcted area, since fibers in these regions depolarized before the subendocardial Purkinje fibers in the region of the infarct. Rapid, repetitive depolarizations then continued at cycle lengths of 100–200 msec without electrical stimulation (Fig. 1A).

Repetitive depolarizations in the noninfarcted regions bordering the infarct of each preparation usually occurred at regular cycle lengths. Depolarization of subendocardial Purkinje fibers in the infarcted region did not always occur at such regular rates and depended on the cycle length of the tachycardia as well as the electrophysiological characteristics of the Purkinje fibers in different areas, i.e., the time course of repolarization, which was markedly prolonged, and the maximum diastolic potential, action potential amplitude, and $V_{\text{max}}$, which were often reduced (9). When the cycle length during tachycardia was sufficiently long (250–300 msec), all regions within the subendocardium of the infarct, except for severely depressed areas, were rapidly activated at the same cycle length as was the adjacent noninfarcted area (Fig. 1B). At these rates, action potential duration of subendocardial Purkinje fibers in both the infarcted region (although markedly prolonged when compared with normal) and the noninfarcted areas shortened sufficiently to allow nearly complete repolarization before each succeeding depolarization. At shorter cycle lengths during spontaneous tachycardia in regions in which Purkinje fiber action potential duration was prolonged, successive rapid depolarizations occurred prior to complete repolarization, resulting in slowly rising action potentials with reduced amplitudes (Fig. 1C).

During more rapid tachycardias, conduction block occurred between subendocardial fibers in the noninfarcted tissue and those in the infarct (Fig. 1D).

As we have described previously, electrophysiological parameters of subendocardial Purkinje fibers in some regions of the infarcted areas were severely depressed. Our criteria for severe depression were as follows: maximum diastolic potential $-70$ mv or...
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less, action potential amplitude 90 mv or less, and $V_{\text{max}}$ 100 v/sec or less. Such severely depressed areas were not present in all preparations isolated from infarcted hearts 20-24 hours after coronary occlusion, although all preparations studied at this time demonstrated some reduction of these parameters. Nondepressed areas of the infarct which were also present had electrophysiological parameters which were not significantly different from normal (9). In those preparations which did contain regions of severely depressed subendocardial Purkinje fibers, the rapid, spontaneous activity was confined to the less depressed areas of the infarct and the adjacent noninfarcted regions. In these preparations, a high degree of conduction block always existed between the spontaneously rapidly depolarizing areas and the regions of severe depression (Fig. 2). Rapid activity was never observed in severely depressed areas within the infarct, possibly because of the inability of Purkinje fibers in these regions to repetitively depolarize at such rapid rates.

Tachycardia was occasionally so rapid in these preparations that successive depolarizations of Purkinje fibers in the noninfarcted areas occurred prior to complete repolarization. As a result, the total amplitude and the rate of depolarization of these fibers were diminished. In several instances, repetitive depolarizations occurred after the Purkinje fibers in the noninfarcted regions had repolarized to only $-60$ to $-65$ mv, resulting in a series of rapid, low-amplitude oscillations (Fig. 2).

Termination of the rapid, repetitive depolarizations occurred in one of two ways. The tachycardia in some instances ceased abruptly without prior indication. At other times, abrupt cessation of the tachycardia was presaged by a change from the constant cycle length during rapid activity to alternating long and short cycle lengths (Fig. 2).

![FIGURE 2](http://circres.ahajournals.org/)

Spontaneously occurring rapid activity in canine myocardium isolated from a heart subjected to anterior descending coronary artery ligation 20 hours before study. The in situ heart before excision demonstrated multiple ventricular ectopic beats and periods of ventricular tachycardia. The records shown were obtained 40 minutes after superfusion of the isolated myocardium was begun. Action potentials demonstrated in the top traces of I and II were recorded from an electrophysiologically depressed subendocardial Purkinje fiber in the infarcted region (apex of anterior interventricular septum) and those in the bottom traces were recorded from a Purkinje fiber in the noninfarcted region (tip of the anterior papillary muscle). IA: Recordings during a period when electrical stimulation activated the subendocardium. IB: Spontaneous rapid activity occurred at a rate of 400/min in the Purkinje fiber in the noninfarcted region but not in the depressed fiber. Note the slowly rising foot of the potential in the depressed Purkinje fiber in the top trace, which may have been due to spontaneous diastolic depolarization or slowed conduction into this region of the infarct and will be discussed in more detail later. IC: Characteristic pattern of depolarization during termination of tachycardia. Note the change in the bottom trace from the constant cycle length to alternation in the cycle length before sudden termination. This change was accompanied by variations in action potential configuration of the depressed fiber in the infarct. II: Another episode of rapid activity in the noninfarcted area is shown at a faster sweep speed. Rapid activity began with a premature depolarization. Repetitive depolarization of the Purkinje fiber in the noninfarcted region became so rapid (850/min) that the fiber was reactivated long before it completely repolarized. Note the periodicity of the pattern of depolarization in the rapidly firing fiber. The cell in the infarcted region fired before each run of rapid activity. Again, this rapid activity did not activate the depressed region.

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We never observed a gradual slowing of the rate of rapid activity before the tachycardia ceased.

In addition to the spontaneously initiated rapid activity described above, tachycardia could be induced by a premature stimulus applied to the noninfarcted region bordering the infarct during periods in which the preparation was either electrically stimulated at the regular cycle length or showed a slow spontaneous rhythm (Fig. 3). Such rapid activity could be induced long after spontaneously occurring tachycardias had subsided. Rapid activity was induced only by premature impulses which conducted into the subendocardial Purkinje fiber network in the infarcted region before these fibers had completely repolarized. Premature impulses which were initiated late in diastole in the noninfarcted region and conducted into the subendocardium of the infarct after Purkinje fibers in this region had almost completely repolarized, did not initiate tachycardia (Fig. 3A). Likewise, premature impulses initiated early during repolarization of Purkinje fibers in the noninfarcted area, which did not conduct into the infarcted area where action potential duration was prolonged, also did not initiate rapid activity. (Fig. 3D).

Spontaneously occurring rapid, repetitive activity was not observed in preparations isolated from noninfarcted hearts or from infarcted hearts after the period of in situ arrhythmias had subsided (3 days to 7 weeks after coronary artery ligation). Also, in preparations isolated at these later times, rapid activity could seldom be induced by premature stimulation.

**FIGURE 3**

Initiation of rapid activity in subendocardial Purkinje fibers with a premature stimulus. The isolated preparation was obtained from a heart subjected to anterior descending coronary artery ligation 24 hours before study. The records shown were obtained 70 minutes after superfusion of the isolated myocardium was begun and after spontaneously occurring rapid activity had ceased. Traces 1 and 2 in each panel show action potentials recorded from Purkinje fibers at the base of the anterior papillary muscle and at the apex of the paraseptal free walls, respectively; both regions were in the infarcted zone. Action potentials in trace 3 of each section were recorded from a Purkinje fiber in the noninfarcted tip of the anterior papillary muscle. External stimuli were applied to the free-running false tendon during a slow spontaneous rhythm. A: Premature impulse (arrow) initiated in diastole of the fiber in the noninfarcted area conducted into the infarcted region and depolarized the Purkinje fibers in traces 1 and 2 after they were nearly completely repolarized. No rapid activity occurred. B: Premature impulse was initiated slightly earlier in diastole of the fiber in trace 3 and activated the subendocardial Purkinje fibers in traces 1 and 2 earlier during repolarization. Rapid spontaneous activity followed this premature impulse. C: Premature impulse was initiated during repolarization of the fiber in the noninfarcted region and elicited only an abortive response in trace 2 and did not depolarize the fiber in trace 1. Rapid activity still followed this premature impulse. D: Premature impulse was initiated still earlier during repolarization of the fiber in the noninfarcted region and did not conduct into the regions of the infarct from which traces 1 and 2 were recorded. No rapid activity occurred.

**FIGURE 4**

Development of spontaneous diastolic depolarization in a subendocardial Purkinje fiber in the infarcted region of a preparation obtained from a heart subjected to left anterior descending coronary artery ligation 24 hours before study. The records shown were obtained 120 minutes after superfusion of the isolated myocardium was begun. Action potentials shown in the top trace in each section were recorded from a depressed Purkinje fiber in the infarct (midparaseptal wall). Action potentials shown in the bottom trace in each section were recorded from a Purkinje fiber at the tip of the anterior papillary muscle (noninfarcted). A: When electrical stimulation was terminated (arrow), spontaneous diastolic depolarization in the fiber in the top trace became obvious and was accompanied by a decrease in upstroke velocity and amplitude of the action potential in the infarct fiber (B). B: The fiber in the infarct (same fiber as in A) was depolarized 300 msec before the fiber in the noninfarcted region. Note that there was no spontaneous diastolic depolarization of the fiber in the noninfarcted region.

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spontaneous impulse initiation occurred in severely depressed subendocardial Purkinje fibers in infarcted areas. This phenomenon became evident after the period of rapid activity had ceased. Such slow spontaneous activity persisted for several hours during superfusion with the oxygenated Tyrode's solution.

Subendocardial Purkinje fibers with severely depressed transmembrane action potential parameters in the infarcted areas demonstrated some spontaneous depolarization during diastole when they were activated by electrical stimulation (9). When stimulation was terminated, the slope of this diastolic depolarization often increased, and spontaneously initiated action potentials occurred in these fibers (Fig. 4). Spontaneous depolarization during diastole never occurred in less depressed subendocardial Purkinje fibers in the infarcted area.

FIGURE 5
Entrance block into a region of severely depressed subendocardial Purkinje fibers in an infarct. The preparation was isolated from a heart subject to left anterior descending coronary ligation 24 hours before study. The in situ heart demonstrated multiple ventricular ectopic beats. The records shown were obtained 180 minutes after superfusion of the isolated myocardium was begun. The top trace in each section was recorded from a severely depressed spontaneously depolarizing Purkinje fiber at the base of the anterior papillary muscle in the infarcted region, the middle trace was recorded from a Purkinje fiber in the noninfarcted tip of the anterior papillary muscle, and the bottom trace was recorded from another depressed Purkinje fiber at the apex of the anterior interventricular septum in the infarcted region. Stimuli were applied to the free-running false tendon. A: The false tendon was stimulated at a cycle length of 640 msec; this procedure activated the Purkinje fibers shown in the middle and bottom traces. The spontaneously depolarizing fiber in the top trace was protected from electrical stimulation by entrance block; there was no temporal relationship between depolarization of this fiber and the driven action potentials in traces 2 and 3. In B, C, and D the rate of electrical stimulation was progressively increased. The rate of depolarization in the top trace remained approximately 60/min, although the rate of activity in the noninfarcted areas was increasing. B: The more rapid activity propagated into the depressed area from which trace 3 was recorded. C and D: Rate-dependent conduction block occurred in this area.
region or in the noninfarcted areas, in these fibers maximum diastolic potential remained at a steady level prior to excitation.

After termination of electrical stimulation, the entire subendocardial region of the isolated preparation (both infarcted and noninfarcted areas) was activated at a slow, spontaneous rate. Severely depressed Purkinje fibers within the infarct that demonstrated spontaneous diastolic depolarization always depolarized 100-200 msec before less depressed regions of the infarct or the bordering noninfarcted areas (Fig. 4). These severely depressed areas were the regions that were activated earliest, suggesting that impulse initiation occurred within these areas of the infarct.

Regions of spontaneously depolarizing Purkinje fibers in the infarct which demonstrated both entrance and exit block were found in four preparations. Subendocardial Purkinje fibers in these areas depolarized spontaneously at constant cycle lengths that were unaffected by the rates at which the surrounding areas were electrically stimulated, even at very long stimulus cycle lengths (Fig. 5). When electrical stimulation of the surrounding regions was terminated, spontaneous activity in the depressed regions continued (Figs. 6, 7). Exit block was often evident after termination of electrical stimulation; the spontaneous activity in the depressed areas did not conduct into surrounding regions which remained quiescent (Fig. 7). In other instances, exit block did not occur after electrical stimulation was terminated. When exit block did not occur, every action potential of the spontaneously depolarizing fibers was followed by excitation of the surrounding myocardium, and the activity occurring in the depressed region preceded activation of the surrounding myocardium by 100-200 msec. The spontaneous rate of the preparation was identical to the rate at which the spontaneously depolarizing fibers were firing during entrance block prior to termination of electrical stimulation (Fig. 6). These characteristics suggest that activity arising in the regions of spontaneously depolarizing Purkinje fibers demonstrating entrance block excited the entire subendocardial region of the infarcted and noninfarcted areas.

In other instances, the relationship between spontaneously firing depressed subendocardial fibers and activation of surrounding regions was not 1:1, and the time interval between depolarization of the depressed and surrounding areas was not constant (Fig. 7). It was therefore not possible to speculate whether the spontaneously depolarizing region in the infarct was the site of impulse initiation for the entire subendocardium.

Purkinje fibers exhibiting spontaneous diastolic depolarization were only rarely encountered in preparations excised 3 days after coronary occlusion and were never found in infarcted preparations studied later than 3 days after occlusion or in preparations isolated from noninfarcted hearts. The potassium concentration in the Tyrode's solution used to superfuse all isolated preparations was 4 mM. This potassium level, in combination with the rapid superfusion rate (25 ml/min) and the saturated O₂ content of the Tyrode's solution, was not conducive to development of spontaneous activity in these preparations (11).

Conduction of Premature Impulses in the Subendocardial Purkinje Fiber Network of Infarcted Preparations.—The conduction of premature impulses in the subendocardial Purkinje fiber network
of the anterior papillary muscle, paraseptal free wall, and anterior interventricular septum of infarcted preparations was studied after the period of rapid arrhythmias subsided and was compared with the conduction of premature impulses in noninfarcted preparations. Premature stimuli were applied through an intracellular microelectrode directly to subendocardial Purkinje fibers in the noninfarcted regions of infarcted preparations (tip of papillary muscle or base of anterior interventricular septum) and in corresponding regions of noninfarcted preparations in an attempt to avoid direct electrical stimulation of underlying ventricular muscle (see Methods).

The distribution of subendocardial Purkinje fiber action potentials with different time courses of repolarization was an important determinant of the characteristics of conduction and sites of conduction block of premature impulses, since this distribution determined the magnitude of membrane potential at which the premature impulses arose and conducted. Myerburg et al. (12) have previously shown that Purkinje fiber action potential duration in noninfarcted hearts increases progressively from the origin of the anterior division of the left bundle branch along the free-running false tendon and reaches a maximum several millimeters from the insertion of the false tendon into the papillary muscle. This region has been referred to as the "gate" (12). In our noninfarcted preparations we determined that action potential duration to 100% repolarization at the region of the gate was 365 ± 24 msec at a stimulus cycle length of 800 msec (mean ± SD in the six preparations). By recording transmembrane action potentials from subendocardial Purkinje fibers at 3-5-mm intervals distal to this site, we determined that, progressing from the tip of the anterior papillary muscle to the apex of the noninfarcted heart, subendocardial Purkinje fiber action potential duration became progressively shorter (Fig. 8). Likewise, Purkinje fiber action potential duration progressively decreased from the base of the heart to the apex in the subendocardial network of the paraseptal free wall and anterior septum. Therefore, in the noninfarcted anterior left ventricle, Purkinje fibers with the longest action potential durations are located on the free-running false tendon of the anterior division of the left bundle branch. Purkinje fibers with the shortest time course of repolarization are located toward the apex of the heart.

As a result of this pattern in noninfarcted hearts, premature impulses initiated by stimulation of subendocardial Purkinje fibers at the tip of the papillary muscle or at the base of the paraseptal free wall or anterior septum encountered Purkinje fibers which were progressively more repolarized as they propagated towards the apex. Premature impulses initiated at the earliest possible time during repolarization of Purkinje fibers at the base of the heart still encountered fibers that were nearly...
FIGURE 8

Distribution of action potential durations of subendocardial Purkinje fibers in a normal, noninfarcted preparation and an infarcted preparation. In each section, the action potentials shown were recorded from the most superficial subendocardial Purkinje fiber at the sites designated on the accompanying photograph of each preparation. In the normal noninfarcted preparation (left), the action potential recorded from the tip of the papillary muscle had the longest time course of repolarization. Action potential duration to 50% repolarization ($APD_{50}$) = 221 msec and $APD$ to 100% repolarization ($APD_{100}$) = 356 msec. Action potential duration progressively decreased from the tip of the papillary muscle and high septum toward the apex of the left ventricle. In the midregion of the anterior papillary muscle $APD_{50}$ = 217 msec and $APD_{100}$ = 322 msec. The shortest action potential was recorded at the apex ($APD_{50}$ = 179 msec and $APD_{100}$ = 290 msec). In the infarcted preparation (right), the top action potential was recorded from the noninfarcted tip of the papillary muscle; action potential duration in this region was normal ($APD_{50}$ = 240 msec and $APD_{100}$ = 360 msec). As the microelectrode was advanced distally across the border into the infarcted region, action potential duration progressively increased. In the mid-region of the anterior papillary muscle $APD_{50}$ = 250 msec and $APD_{100}$ = 400 msec. Note that the action potential recorded from the apex of the left ventricle was more than 100 msec longer than the action potential recorded from the tip of the papillary muscle ($APD_{50}$ = 265 msec and $APD_{100}$ = 500 msec).

completely repolarized by the time they had propagated two-thirds of the distance towards the apex (Fig. 9). Therefore, conduction delay and block of early premature depolarizations always occurred in Purkinje fibers in the vicinity of the intracellular stimulating electrode. The repolarization time course was longest in these Purkinje fibers. Conduction block never occurred toward the apical regions, since action potential duration in these areas was always less than it was in the stimulated area.

The pattern of conduction of premature impulses and the sites of conduction block of these impulses in the subendocardial Purkinje fiber network of infarcted preparations differed from those described for the noninfarcted subendocardial network. In infarcted preparations action potential durations of Purkinje fibers progressively increased from the base of the heart to the apex in all regions (Fig. 8). This pattern resulted from the marked increase in action potential duration of Purkinje fibers in the infarcted region (9). As a result, premature impulses initiated either early in diastole or before complete repolarization of subendocardial Purkinje fibers in noninfarcted tissue at the tip of the papillary muscle or the base of the paraseptal free wall or anterior septum conducted extremely slowly once they propagated into the infarcted area (Fig. 9). Such slow conduction occurred because the time course of repolarization of subendocardial Purkinje fibers in the infarcted area was 100–200 msec longer than it was in the surrounding noninfarcted areas.
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Conduction of premature impulses in the subendocardial Purkinje fiber network of a normal (left) and an infarcted (right) preparation. Premature stimuli were delivered through an intracellular microelectrode to a subendocardial Purkinje fiber on the tip of the anterior papillary muscle. In each section, the top trace is the transmembrane action potential of the prematurely stimulated fiber on the tip of the papillary muscle. The bottom trace was recorded from a subendocardial Purkinje fiber at the apex of the left ventricle. The arrows indicate the premature response at the apex. For the infarcted preparation, the apical Purkinje fiber was located in the infarcted region, but the Purkinje fiber at the tip of the papillary muscle was not. Note that the apical Purkinje fiber had a shorter action potential duration than did the fiber at the tip of the papillary muscle in the normal preparation and a longer action potential duration than did the fiber at the tip of the papillary muscle in the infarcted preparation. For the normal preparation a premature impulse initiated after complete repolarization of the proximal fiber excited the fiber in the apex after a delay of only 10 msec (top section). In the middle section, an earlier premature impulse still conducted to the apex with a delay of only 10 msec. A still earlier premature stimulus (bottom section) excited the Purkinje fiber on the tip of the papillary muscle early during repolarization, but conduction delay to the apex was still not substantial. In the infarcted preparation, a premature impulse initiated after complete repolarization of the proximal fiber (top section) conducted to the fiber in the apex with a delay of 12 msec. In the middle section, an earlier premature impulse conducted into the infarct to the apex with substantial delay; the fiber in the apex was excited nearly 50 msec after the proximal fiber. A still earlier premature stimulus (bottom section) excited the fiber on the tip of the papillary muscle and conducted to the apex with a conduction time of 88 msec. Conduction delays of this magnitude were never seen in the subendocardial Purkinje fibers of noninfarcted preparations.

Furthermore, action potential durations in the infarct increased in the same direction as propagation of the premature impulse, i.e., from base to apex (Fig. 8). Premature impulses conducting into these subendocardial areas therefore encountered Purkinje fibers which were progressively less repolarized and therefore probably more refractory.

In addition to being prolonged, the time course of repolarization of subendocardial Purkinje fibers in the infarcted region was strikingly inhomogeneous with action potentials in adjacent regions having markedly different durations. As a result, conduction of premature impulses through the subendocardial Purkinje fiber network of the infarct was not only slowed but was also inhomogeneous, that is, conduction of premature impulses from the base to the apex of the preparation was slower in regions with the longest action potential durations and more rapid in closely adjacent regions with shorter action potential durations. Consequently, conduction block of early premature impulses occurred in some areas of the infarct, but conduction through other infarcted regions still proceeded slowly. This observation was documented by experiments in which microelectrode recordings were obtained from multiple sites during the propagation of premature impulses (see Methods). The results of one such investigation are demonstrated in Figure 10. In this experiment, a premature impulse initiated late during repolarization of a Purkinje fiber in the noninfarcted region at the base of the septum conducted into the infarct and depolarized Purkinje fibers toward the apical portions of the septum and the anterior papillary muscle with very little delay (Fig. 10A). Premature impulses initiated earlier during repolarization of the fiber in the noninfarcted region conducted to the apical portion of the papillary muscle extremely slowly and depolarized the fiber at this site after considerable delay (Fig. 10B and C, trace 4). These early premature impulses, however, blocked as they propagated toward the apex of the septum, as indicated by the low-amplitude depolarization of a fiber in this region (Fig. 10B and C, solid arrows on trace 3).

In four separate experiments such as the one illustrated in Figure 10, in the regions of the infarct where conduction block of early premature impulses occurred, subendocardial Purkinje fibers often demonstrated an unstimulated depolarization (Fig. 10B and C, open arrows on traces 2 and 3) which followed the depolarization resulting directly from the premature stimulation (Fig. 10B and C, solid arrows on traces 2 and 3). For each of these
FIGURE 10

Inhomogeneous conduction of premature impulses in the subendocardial Purkinje fiber network of an infarcted preparation 24 hours after coronary occlusion. **Top:** Schematic representation of the preparation. Light area indicates infarcted region. PM = papillary muscle, FT = false tendon, and S = septum. The numbers in this drawing denote the locations of sites from which subendocardial Purkinje fiber action potentials were recorded during premature stimulation. Two stationary microelectrodes were utilized to record action potentials from sites 1 and 4 throughout the experiment. Premature stimuli (at variable coupling intervals with the basic stimulus) were applied through the intracellular microelectrode at site 1. The response resulting directly from this premature stimulus is indicated by the solid arrows on each trace in A, B, and C. At each coupling interval a third (roving) microelectrode was utilized to record action potentials from sites 2, 3, and 5-9 (see Methods). These action potentials were displayed simultaneously with those recorded from sites 1 and 4. By this method, the relative sequence of activation of these nine recording sites by the premature impulse was determined. **Bottom:** For the records shown in A, B, and C, action potentials were recorded from sites 1, 2, and 4 during premature stimulation at three given coupling intervals and then from sites 1, 3, and 4 at the same coupling intervals. The records shown are a composite of the recordings obtained at these four sites. In each of these sections, the numbered traces were recorded from the correspondingly numbered sites on the accompanying diagram of the preparation. A: Premature stimulus was applied through the microelectrode at site 1 320 msec after the basic impulse. This premature impulse conducted into the infarct to sites 2, 3, and 4 and depolarized the Purkinje fibers at these sites nearly simultaneously. B: Coupling interval was 300 msec. This premature impulse appeared to block near recording site 3, as indicated by the low-amplitude depolarization at this site (solid arrow on trace 3). This premature impulse, however, conducted slowly to site 4 without blocking, as indicated by the delayed depolarization at this site. Note also the additional depolarizations at sites 3, 2, and 1 (open arrows on traces 3, 2, and 1) which followed the response at site 4. C: Coupling interval was reduced to 280 msec. This premature impulse appeared to block before reaching site 3 (low-amplitude deflection indicated by solid arrow on trace 3) but still conducted slowly to site 4 without blocking. Now note the additional depolarizations at sites 3, 2, and 1 (open arrows on traces 3, 2, and 1) which followed the response at site 4. See Discussion and Figure 11 for a possible interpretation of these unstimulated depolarizations.

four experiments similar unstimulated depolarizations also occurred repeatedly in the noninfarcted region of the preparation after premature stimulation at a particular coupling interval with the basic drive stimulus (Fig. 10C, open arrow on trace 1). Although the origin of these unstimulated depolarizations was not certain, one possible interpretation is that they resulted from reentry within the
Discussion

If ligation of the canine left anterior descending coronary artery is accomplished in two stages as described by Harris (10), the dogs sometimes develop transient ventricular arrhythmias within minutes after complete occlusion of the artery but survive this early arrhythmic stage. Subsequently they develop more sustained arrhythmias 20-24 hours after coronary occlusion (10). This sequence of events may be comparable in some ways to the pattern of myocardial infarction arrhythmias in humans; early arrhythmias may occur within minutes of the onset of symptoms and may also persist for several days thereafter (15-18).

The electrophysiological mechanisms and origins of these ventricular arrhythmias are still obscure. Many investigators have proposed that anoxia or hypoxia of cardiac muscle in the area deprived of its blood supply may be one causative factor (1, 2, 4, 19). Previous electrophysiological studies on the intact heart have demonstrated significant changes in the characteristics of extracellular electrograms recorded within the ischemic region immediately after coronary artery occlusion which are associated with and may be responsible for early ventricular arrhythmias (5, 20, 21). However, 20-24 hours after occlusion, the time at which our studies were performed, intramural electrical activity in the infarcted region has ceased, although ventricular arrhythmias still occur (9). It is logical to assume that cardiac arrhythmias occurring 20-24 hours after coronary artery occlusion must arise in cells capable of generating electrical activity; therefore, they may arise at the border zones between infarcted and noninfarcted myocardium and possible mechanisms for production of these arrhythmias have been suggested (1, 22-25). As we have previously demonstrated, the subendocardium of an infarct is covered by a blanket of viable Purkinje fibers (9). Our in vitro observations on the electrophysiological events in this subendocardial Purkinje network suggest that it may participate in the genesis of ventricular arrhythmias in vivo. Furthermore, the occurrence of electrophysiological phenomena such as those described in the present study suggests that this isolated preparation may be useful in the continuing investigation of those electrophysiological properties of cardiac cells which are responsible for arrhythmias in the intact infarcted heart.

Rapid, repetitive depolarization of subendocardial Purkinje fibers in preparations removed from infarcted hearts with cardiac arrhythmias occurred during the first 60 minutes of superfusion. Such sustained rapid activity was never observed in preparations isolated from nonarrhythmic hearts.
whether they were noninfarcted hearts or hearts removed 3 days to 7 weeks after coronary artery occlusion, at which time arrhythmias were no longer present. Also, rapid activity of this type has not been reported for bundles of Purkinje fibers isolated from noninfarcted hearts, although such bundles have been used for numerous electrophysiological studies. Our observations suggest, therefore, that this rapid activity may be one cause of arrhythmias in the in situ infarcted heart. The reason for cessation of rapid spontaneous activity after approximately 60 minutes of superfusion is uncertain. If substances released from the necrotic infarcted myocardium are responsible for the tachyarrhythmias, they may be washed out after this period of superfusion (22, 26, 27). This question requires further investigation. However, even after cessation of spontaneous repetitive activity, repetitive discharge of a much shorter duration could be induced in the subendocardial Purkinje fiber network of the infarcted area by premature stimulation.

Rapid, repetitive depolarizations of subendocardial Purkinje fibers began with a premature impulse, either occurring spontaneously or electrically induced, which depolarized the Purkinje fibers in the infarcted region well before complete repolarization. This characteristic suggests a possible relationship to the occurrence of paroxysmal ventricular tachycardia in the in situ heart after myocardial infarction (28). Paroxysmal ventricular tachycardia is often initiated by a spontaneously occurring ventricular premature depolarization occurring shortly after the T wave (29). This premature depolarization should coincide with the relative refractory period of Purkinje fibers with prolonged action potentials in the subendocardial network of the infarcted region.

The mechanism for the maintenance of the rapid activity in our isolated preparations has not been determined. It seems unlikely that the rapid activity was due to spontaneous diastolic depolarization, since, during rapid activity, subendocardial Purkinje fibers were repetitively depolarized before complete repolarization. The rapid rate would hardly permit time for spontaneous diastolic depolarization.

The events which occurred in our isolated preparations during conduction of premature impulses into the infarct, such as those illustrated in Figure 10, may possibly be related to the mechanisms which initiated or sustained rapid, repetitive activity after premature activation. In the experiment depicted in Figure 10, a stimulated early premature impulse propagating from the noninfarcted region into the infarct was followed consistently by an unstimulated impulse propagating in the opposite direction. The sequence of activation of multiple recording sites during this experiment and the events which occurred at each of these sites suggest that reentry may have taken place. A late premature impulse, initiated in the noninfarcted region at a coupling interval of 320 msec with the basic stimulus, conducted into the infarct and depolarized Purkinje fibers in the apical portions of the septum and papillary muscle (Fig. 10A). The activation sequence of our nine recording sites could have resulted from propagation of the premature impulse over a pathway such as the one illustrated diagrammatically in Figure 11A. When the coupling interval was reduced to 300 msec the premature impulse appeared to block near recording site 3 on the septum, as indicated by the low-amplitude depolarization, but it still conducted to site 4 on the papillary muscle (Fig. 10B). The unstimulated depolarizations at sites 3 and 2 on the septum which occurred at this coupling interval (Fig. 10B, open arrows) could have resulted from the premature impulse propagating past site 4 retrograde up the septum to sites 3 and 2 and then blocking near this latter site (Fig. 11B). Finally, when the coupling interval was reduced to 280 msec, the premature impulse propagating down the septum appeared to block before reaching site 3 (Fig. 10C, the low-amplitude depolarization indicated by the solid arrow on trace 3 is even smaller than the one in Fig. 10B). This premature impulse, however, again conducted to site 4 on the papillary muscle without blocking. At this particular coupling interval, the unstimulated depolarizations at sites 3, 2, and 1 (Fig. 10C, open arrows on traces 3, 2, and 1) which followed the depolarization at site 4 could have been caused by conduction of the premature impulse past site 4 retrograde up the septum exciting, in turn, the fibers at sites 3, 2, and finally 1 as a reentrant impulse. This possible pathway (Fig. 11C) would be compatible with the records shown in Figure 10C. A similar sequence of events occurred during premature stimulation in three other experiments. Although these data are consistent with the occurrence of reentry, such a phenomenon can be documented conclusively only by measurement of the excitation wave throughout its entire course. Since this procedure was not possible in the present study, interpretations other than the one we have offered cannot be excluded.
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However, if the phenomenon we observed was, in fact, reentry, its occurrence was probably made possible by the extremely long conduction delays and the conduction block of premature impulses as they propagated into infarcted regions where subendocardial Purkinje fiber action potential duration was markedly prolonged. Conduction delay and block did not occur when premature impulses propagated from base to apex in the normal left ventricle. Continuous reentry due to such a mechanism might occur under certain circumstances. Whether this reentry is in any way responsible for the rapid tachyarrhythmias which we observed both in situ and in vitro remains to be determined.

In addition to rapid activity, subendocardial Purkinje fibers in the infarcted region demonstrated other electrophysiological characteristics that might be instrumental in the genesis of arrhythmias after infarction. Severely depressed subendocardial Purkinje fibers developed marked spontaneous diastolic depolarization. Regions demonstrating this property appeared to function as pacemakers in the absence of electrical stimulation. However, since spontaneous diastolic depolarization need not indicate automaticity, it is difficult to determine whether such fibers are truly automatic (30). Such spontaneous diastolic depolarization never occurred in Purkinje fibers in noninfarcted regions under our experimental conditions, i.e., rapid superfusion with oxygenated Tyrode's containing 4 mm potassium. Therefore, this spontaneous diastolic depolarization was not due to anoxic conditions at the time of study. The mechanism by which myocardial infarction leads to the development of spontaneous diastolic depolarization also requires further study.

Spontaneous impulse initiation of this type in the subendocardial Purkinje network of the infarcted region may result in ventricular ectopic beats or rhythms in the in situ heart. Under the conditions utilized in our study the spontaneous firing rate of these subendocardial Purkinje fibers was slow (50-70/min). This type of impulse initiation is consistent with certain characteristics of nonparoxysmal ventricular tachycardia or idioventricular rhythm after acute myocardial infarction (29, 31-34). This arrhythmia is relatively slow in rate (60-100/min) and manifests itself during long sinus cycle lengths or sinus bradycardia. Therefore, it is probably an escape rhythm and may be due to an increased automaticity of subendocardial Purkinje fibers in the infarcted region.

Entrance and exit block associated with regions of spontaneously depolarizing subendocardial Purkinje fibers in the infarcted area were also found. Such areas constituted typical parasystolic foci (35-37). Ventricular parasystole occurs clinically after acute myocardial infarction (38). Although the existence of such foci of spontaneously firing cardiac fibers with entrance and exit block has been postulated from electrocardiographic studies, direct electrophysiological demonstration utilizing microelectrode recordings has previously been lacking. Our microelectrode recordings have demonstrated the occurrence of such foci in vitro after experimental myocardial infarction.

No attempt was made in the present study to define the mechanisms underlying entrance and exit block in these foci of spontaneously active Purkinje fibers. However, the demonstration that such areas exist in infarcted myocardium suggests that this preparation can be used to investigate these mechanisms and the manner in which they are influenced by pharmacological agents.

References


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