Electrophysiological Properties of Canine Purkinje Cells in One-Day-Old Myocardial Infarction

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ABSTRACT

Electrophysiological mechanisms which underlie the ectopic ventricular beats occurring 1 day after occlusion of the anterior descending coronary artery of dogs were explored. In intact dogs, bipolar electrograms were recorded in the infarcted and normal zones. Both Purkinje and ordinary myocardial potentials were recorded from the endocardial surface of the normal zone, but only Purkinje potentials were recorded from the infarcted zone. The Purkinje potentials in the infarcted zone were diminished in amplitude and rapidity. The threshold for endocardial pacing was higher in the infarcted zone than it was in the normal zone. The refractory periods of Purkinje cells were longer in the infarcted zone than they were in the normal zone. Excised specimens of endocardial surface containing the infarcted zone were superfused with Tyrode's solution. No ordinary myocardial cells were electrically active at the endocardial surface of the infarcted zone. Purkinje cells showed diminished resting and action potentials, reduced upstroke velocity, enhanced automaticity and phase 4 depolarization, and long action potentials with prolonged phase 3. Excitability was depressed within the infarcted zone. During superfusion, even with Tyrode's solution poor in oxygen and free of dextrose, Purkinje cells recovered toward normal with respect to amplitude of resting and action potentials, upstroke velocity, automaticity, and excitability. The prolonged action potentials persisted. Repetitive firing was easily elicited within the infarcted zone. Various types of pacemaker activity were detected within the infarcted zone. Thus Purkinje cells in the infarcted zone showed distinctive alterations in electrophysiological properties which were probably related to deleterious factors that accumulated in that zone. It is likely that the arrhythmia which occurred in the intact dogs at this time originated within the altered Purkinje cells of the infarcted zone.

KEY WORDS prolonged action potentials enhanced automaticity recovery hypoxia working myocardium reentry pacemaker

Ectopic beats of ventricular origin constitute the most common disturbance in rhythm after myocardial infarction in man (1, 2). In the dog, a brief period (up to 20 minutes) of ectopic ventricular activity which often culminates in ventricular fibrillation ensues after abrupt occlusion of the anterior descending coronary artery (3). Following this initial burst of ectopic activity, there is a relatively quiet period (6-12 hours) when sinus rhythm predominates. Then a second surge of ectopic ventricular activity occurs which may last for several days (4). The important mechanism for the initial arrhythmia appears to be reentry resulting from slow conduction in ischemic myocardium (5-8). However, certain observations have suggested that the later arrhythmia is a consequence of enhanced automaticity within the infarct (9). To investigate the cellular mechanisms involved in this phase of arrhythmia, we recorded intracellular and extracellular potentials from the excised endocardial surface on the day after occlusion of the anterior descending coronary artery in the dog. Also we recorded Purkinje and myocardial potentials from the infarcted zone of the intact heart before excision. We found that there was no electrical activity from ordinary myocardial cells at the endocardial surface within the infarct. However, the recordings from Purkinje cells disclosed distinctive alterations in electrophysiological properties.

Methods

Through a left thoracotomy the anterior descending coronary artery of 29 mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg, iv) was ligated in one or two stages as described by Harris (4). There
were no systematic differences in the data collected after one- or two-stage ligation so the results were combined. The following day (16-30 hours after occlusion) a left thoracotomy was again performed under sodium pentobarbital anesthesia and the heart was exposed. Bipolar recordings were made from the endocardial and epicardial surfaces of the ischemic and normal regions. On the electrograms from the endocardial surface, both Purkinje and myocardial potentials could be distinguished. To obtain these recordings, the tips of fine Teflon-coated stainless steel wires (0.003 inches in diameter) were inserted via a 25-gauge needle into the left ventricular cavity and hooked gently into the endocardium. Purkinje potentials were distinguished from myocardial potentials by their earlier occurrence and their more rapid inscription. For threshold determinations, endocardial sites were paced through the fine wires at rates of 160-180/min with rectangular pulses 2 msec in duration. In 6 dogs, the wires were inserted before the occlusion into multiple sites within and outside the zone of distribution of the left anterior descending coronary artery. This zone of distribution was estimated from the pattern of the vessels on the epicardial surface. The wires were left in place for 12-18 hours after occlusion, and recordings were made at intervals.

After these in vivo recordings had been made, the heart was excised and specimens which included normal and infarcted ventricle were pinned with the endocardial surface upwards. The specimens were superfused with warmed (35-37°C), aerated (95% O_2-5% CO_2) Tyrode's solution of the following millimolar composition: Na^+ 151.1, K^+ 4.05, Ca^{2+} 1.35, Mg^{2+} 0.5, Cl^- 131.25, \( HCO_3^- \) 24.0, \( HPO_4^{2-} \) 1.8, and dextrose 5.5. In some experiments the solutions were equilibrated with 95% N_2-5% CO_2 or with 20% O_2-75% N_2-5% CO_2. Sometimes dextrose was omitted from the solution. In some experiments practolol was included in the Tyrode's solution in a concentration of 1 mg/liter, this concentration blocked the chronotropic effect of 10^{-4}M epinephrine.

The excision and dissection were performed with haste so that recordings could be made in most cases within 10 minutes after excision of the heart. Special care was taken to minimize mechanical or manual trauma to the infarcted area, especially the endocardium, during the excision and dissection. The infarcted zone on the endocardial surface was visibly distinguishable from the surrounding normal zone as a clearly demarcated, irregularly circular area which was pale, mottled, and ecchymotic. It was 2-4 cm in diameter and occupied the apex and the anterior and lower portions of the septum and free wall, including the base of the anterior papillary muscle. In this region of uninfarcted hearts peripheral Purkinje fibers ramify profusely over the endocardium, and Purkinje-muscle couplings are plentiful. The pinned preparation usually included all of the visibly infarcted endocardial surface and a surrounding rim (1-2 cm in width) of normal tissue. Sites within this normal zone were monitored with intracellular and extracellular recordings at frequent intervals throughout the experiment. In some experiments another specimen containing only normal endocardial surface from the posterior and inferior region of the left ventricle or the apical region of the right ventricle was included in the perfusion chamber for control. Also for control, potentials were measured in preparations from four uninfarcted hearts, which included apical regions comparable to the regions employed in the infarcted hearts. At the time of dissection of the hearts which were studied in vivo, we verified the location of the tips of the fine wires used for the in vivo recordings.

For the recordings in the intact dog, the signals were led from the wires into differential amplifiers (Electronics for Medicine, EEP) and recorded on an oscilloscope recorder (Electronics for Medicine, DR-8). Signals were filtered at low and high limits of 0.1 and 2000 cycles/sec or 400 and 2000 cycles/sec. From the isolated tissues, extracellular potentials were recorded with bipolar electrodes of Teflon-coated stainless steel wires (0.003 inches in diameter) led into differential operational amplifiers. Intracellular potentials were recorded with machine-pulled microelectrodes which had a tip resistance of 10-40 megohms when they were filled by boiling with 3M KCl. The microelectrodes were connected through a silver-silver chloride interface to the input of a high-impedance (greater than 10^{12} ohms) amplifier with a low gain (3X) and an adjustable negative capacitance (Bioelectric NF1). The indifferent electrode in the chambers was an agar bridge of 3X KCl with a silver-silver chloride interface. The outputs of the first-stage amplifiers were led into a plug-in amplifier (Tektronix 3A74) in a dual-beam oscilloscope (Tektronix 565). The rate of rise of action potentials was obtained with a resistance-capacitance differentiating circuit that had a linear response up to 1000 v/sec. Continuous calibration was obtained using the attenuated sweep-generating voltage of an oscilloscope led into the bath through the indifferent electrode. Voltage calibration for intracellular recordings was accomplished using a calibrator voltage (100 mv) inserted across a 100-ohm resistance between the indifferent electrode and ground. The oscilloscope traces were photographed on 35-mm film (Grass C4N).

**Results**

In vivo, all the infarcted dog hearts manifested rapid (120-160/min) ectopic ventricular activity with variable QRS configuration. If the ectopic ventricular activity was masked by sinus tachycardia, it could be uncovered by vagal-induced sinus arrest. Endocardial recordings from the infarcted zone showed small (less than 1.0 mv) slow deflections which usually lasted throughout the QRS complex. These deflections were attributed to activity of relatively distant myocardium (extrinsic deflections). Often, superimposed on these slow deflections were more rapid deflections of varying amplitude (up to 1.0 mv). During sinus rhythm these deflections usually occurred within an interval of 5-20 msec after the onset of the QRS complex. In contrast, in the normal zone, electrograms were...
usually composed of two rapid deflections. The earlier, more rapid one was up to 2 mV in amplitude; this deflection was attributed to Purkinje fibers. In recordings from the upper half of the septum, this early rapid deflection preceded the onset of the QRS complex by 5–15 msec. In all experiments in which rapid spikes were attributed to Purkinje fibers, the wire tips were observed to be in intimate contact with visible strands of conduction tissue after the heart was excised. The later deflection was larger (up to 10 mV) but less rapid. It was attributed to myocardial cells. In some recordings a potential was recorded between the Purkinje and the myocardial spike (Fig. 1, NZ trace). This potential probably represents injury current from the Purkinje fibers; it often disappeared with time. A set of electrograms recorded from the infarcted and normal zones along with three leads of the electrocardiograph (ECG) are shown in Figure 1. The Purkinje spikes in the infarcted zone are smaller than those in the normal zone in this particular set of recordings. In general, the maximum amplitude of Purkinje spikes recorded in the infarcted zone (1.0 mV) was less than that of spikes recorded in the normal zone (2.0 mV). However, the variable proximity of the wire tips to Purkinje strands resulted in great variability in the amplitudes of Purkinje spikes, in both the infarcted and the normal zone. Consequently, for more accurate comparison, recordings were made from the same sites before and after occlusion. Purkinje potentials within the infarcted zone consistently (14 out of 14 sites) lost amplitude and rapidity shortly (within 5 minutes) after occlusion. They remained diminished throughout the period of recording (12–18 hours), which extended into the later phase of arrhythmias. In contrast, the potentials from 6 sites in the normal zone did not vary in amplitude by more than 20% of the control values throughout the period of recording after occlusion. Sites in the border zone were variable. Recordings taken from the same sites in the infarcted, border, and normal zones before and 17 hours after occlusion are shown in Figure 2. In six experiments, thresholds for pacing at endocardial sites within the infarcted zone were high compared with those at endocardial sites in the normal zone.
A wide range of thresholds was encountered in the infarcted zone, including inexcitable sites. Since there were inexcitable sites, i.e., infinite threshold current, a mean could not be calculated for the infarcted zone. However, 32 of 36 sites in the infarcted zone had thresholds at least two times greater than the mean for the normal zone in the same dog (normal zone means were in the range of 0.2 to 0.4 mA).

Soon after isolation, most infarcted preparations demonstrated rapid, irregular firing at rates up to 300/min. Usually within the first 15 minutes after isolation the spontaneous firing rate became regular. The mean ± SD of the firing rate was 70 ± 6 beats/min 0.5 hours after isolation. The firing rate of similar uninfarcted preparations under the same conditions of superfusion was 4 ± 2 beats/min. This rapid firing rate was only slightly affected by practolol—the mean rate diminished from 70 to 58 beats/min.

Immediately after isolation electrograms at some sites in the infarcted zone recorded slow, diminished potentials; at other sites nothing was recorded. A representative group of electrograms recorded from the infarcted zone immediately after isolation is shown in Figure 3A. For preparations to be acceptable, the extracellular and intracellular potentials recorded from the normal zone immediately after isolation had to be normal, i.e., comparable to those recorded from uninfarcted preparations of isolated endocardium. In 1 of 11 preparations, the potentials recorded from the normal zone were abnormal. It was assumed that the trauma of excision and isolation had been excessive in this experiment, and the results were discarded. In all preparations, intracellular recordings from cells in the infarcted zone showed low resting potentials. Cells nearer the border of the infarcted zone generally had greater resting potentials than did those more toward the interior. Examples of action potentials recorded from the infarcted zone soon after isolation are illustrated in Figures 4A, 5A, and 6A.

Initially (Fig. 4A–C) the rate of phase 4 depolarization was invariably greater in the infarcted zone than it was in the normal zone. In time, the spontaneous rate of firing of the preparations diminished. Concurrently, the rate of diastolic depolarization of cells within the infarcted zone decreased, approaching that of cells in the normal zone. In the experiment illustrated in Figure 4, 10 hours were required for the rate of diastolic depolarization of most sites in the infarcted zone to become similar to that of sites in the normal zone (Fig. 4D). After 4 hours of superfusion, the mean firing rate of the preparations was 40 ± 4 beats/min, which is significantly different from the mean firing rate in the first hour (P < 0.01).

In all preparations the cells in the infarcted zone underwent a process of recovery characterized by a gain in the amplitude of resting and action potentials and an augmentation of maximum

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**FIGURE 3**

Electrograms recorded sequentially from the infarcted (IZ) and normal (NZ) zones of an excised specimen of left ventricular endocardium. A: Recorded 8 minutes after excision. B: Recorded 26 minutes after excision. C: Recorded 36 minutes after excision. Purkinje (P) and myocardial (M) potentials were recorded from all sites in the normal zone at all times. The normal zone electrograms in A, B, and C are recordings from three different sites in the normal zone. Initially nothing was recorded from the three infarcted zone sites, but Purkinje potentials appeared later and increased in amplitude during superfusion.

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Sequential comparison of action potentials of Purkinje fibers in the infarct zone (IZ) and the normal zone (NZ). A: Recorded 10 minutes after excision of the heart. B: Recorded from the same cells 1.5 hours after excision. C: Recorded from the same cells 10 hours after excision. D: Recorded from the same cells 12 hours after excision. E: Recorded at the same time as E but at a higher sweep speed to better illustrate the morphology of the action potentials. One trace was inadvertently moved, artifactually changing the relationships of the resting potentials of the two cells. Consequently, the zero line has been omitted in F, which is shown only to illustrate the relative durations of the action potentials. In all sections except E the specimens were beating spontaneously. In the first half of E the specimens were driven at a rate of 60/min; the driving stimulus was turned off midway through E to show the rate of diastolic depolarization during a longer interval. Time calibrations are shown below each section.

Soon after isolation the bizarre action potentials were sometimes difficult to characterize, since they lacked a configuration typical of canine Purkinje or ventricular myocardial cells. However, all cells in the infarcted zone manifested diastolic depolarization, and in time during superfusion action potentials in the infarcted zone assumed a configuration suggestive of Purkinje fiber origin (sharp spikes ending phase 0, comparatively linear phase 2, comparatively slow phase 3). At no time were transmembrane potentials characteristic of ventricular myocardial fibers observed in the infarcted zone (blunt spike ending phase 0, comparatively rounded phase 2, comparatively rapid phase 3, and constant phase 4). The rate of recovery was variable among preparations and within the infarcted zone. In general, the cells near the border of the infarcted zone recovered sooner. Resting and action potentials of a Purkinje cell in various states of recovery are illustrated in Figure 5. In most preparations after 2 hours of superfusion there was little difference between most of the cells within the infarcted zone and cells within the normal zone with respect to amplitude of resting and action potentials and Vmax. Table 1 contains mean values for these parameters during early and later stages of superfusion, along with values for sites in the infarcted zone.

In time, as the cells in the infarcted zone recovered full amplitude, they also recovered normal excitability. The thresholds for stimulation in the infarcted zone in vitro were initially high, but they consistently decreased to values equivalent to those in the normal zone with time. Because inexcitable sites were frequently found, mean values for the threshold were not calculated for the infarcted zone. In the first 0.5 hours after isolation, all of the 23 sites tested within the infarcted zone in five preparations had thresholds at least twice the threshold for sites in the normal zone in the same preparation. In contrast, after 3 hours of superfusion, 27 of 32 sites within the infarcted zone had thresholds no more than one and a half times the threshold found for sites in the normal zones in the same preparation.

This recovery process also manifested itself as an increase in amplitude and rapidity of the spikes on the electrograms recorded from the endocardial surface, as illustrated in Figure 3B and C. The electrograms recorded in the infarcted zone always showed single spikes; they never showed the slower
spikes typical of myocardial activity. In contrast, the recordings from the normal zone always showed a composite electrogram containing rapid (1-3 msec) Purkinje spikes and slower (6-10 msec) myocardial spikes.

Recovery of Purkinje cells within the infarcted zone did not depend on dextrose or high oxygen tension ($P_0_2$) in the superfusate. Figure 6 illustrates a representative experiment in which dextrose-free solution equilibrated with 95% O$_2$-5% CO$_2$ was employed throughout the isolation and superfusion. As in all other preparations, the cells within the infarcted zone were initially depressed (A). Recovery occurred during 85 minutes of superfusion, while the $P_0_2$ of the perfusate was 41 mm Hg. Recovery was observed in each of three other preparations superfused with dextrose-free solution at $P_0_2$ values between 40 and 50 mm Hg.

Purkinje cells within the infarcted zone had prolonged action potentials. This distinctive feature was often detected within the infarcted zone immediately after isolation when Purkinje cells were depressed. It was most striking after the Purkinje cells within the infarcted zone had recovered full amplitude of resting and action potentials. Figure 7 shows a comparison between fully recovered cells in the infarcted zone and cells in the normal zone. The duration of the action potentials in the infarcted zone was most frequently in the range of 500 to 600 msec at a driving cycle length of 800-1000 msec. Mean values are shown in Table 1, along with values for sites in the normal zone. The values for the normal zone were taken from the rim of normal zone surrounding the infarct and from regions in uninfarcted hearts corresponding to the region occupied by the infarcted zone in the infarcted heart to avoid the systematic differences related to different locations in the normal conduction system. This prolongation could not be reproduced in vitro by superfusion of normal Purkinje fibers for 24 hours with Tyrode’s solution equilibrated with 20% O$_2$-75% N$_2$-5% CO$_2$ to more closely approximate the $P_0_2$ of left ventricular chamber blood.

Action potentials of cells within the infarcted zone remained prolonged even after protracted superfusion (up to 14 hours). Thus, the usual sequence for Purkinje cells in the infarcted zone was early recovery of full amplitude of resting and

<table>
<thead>
<tr>
<th>Time</th>
<th>Zone</th>
<th>Resting potential (mv)</th>
<th>Action potential amplitude (mv)</th>
<th>$V_{\text{max}}$ (v/sec)</th>
<th>Action potential duration* (msec)</th>
</tr>
</thead>
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<tr>
<td>0-60</td>
<td>Infarcted</td>
<td>58 ± 13†</td>
<td>45 ± 21†</td>
<td>36 ± 22†</td>
<td>441 ± 59†</td>
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<tr>
<td></td>
<td>Normal</td>
<td>83 ± 6</td>
<td>111 ± 18</td>
<td>400 ± 45</td>
<td>333 ± 36</td>
</tr>
<tr>
<td>60-120</td>
<td>Infarcted</td>
<td>68 ± 10†</td>
<td>75 ± 27†</td>
<td>110 ± 44†</td>
<td>304 ± 97†</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>85 ± 6</td>
<td>117 ± 13</td>
<td>420 ± 42</td>
<td>334 ± 40</td>
</tr>
<tr>
<td>120+</td>
<td>Infarcted</td>
<td>80 ± 12†</td>
<td>106 ± 16†</td>
<td>366 ± 58†</td>
<td>347 ± 107†</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>87 ± 6</td>
<td>119 ± 12</td>
<td>418 ± 50</td>
<td>342 ± 47</td>
</tr>
</tbody>
</table>

All values are means ± sd.

*Action potential duration was measured to 100% repolarization at a driving cycle length of 800-1000 msec.
†Difference between the normal zone and the infarcted zone mean for the same time interval is significant ($P < 0.02$).
‡Difference between the infarcted zone mean at 120+ minutes and that at 0-60 minutes is significant ($P < 0.02$).

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Prolongation of the duration of the action potentials of Purkinje fibers in the infarcted zone.

A: Action potential recorded from a Purkinje fiber in the normal zone 2 mm from the border of the infarct.

B: Action potential recorded at the border of the infarct.

C: Action potential recorded from a Purkinje fiber within the infarct 3 mm from the border. The differentiated upstrokes are shown on the bottom traces. These action potentials were all recorded from the same small peripheral strand of conduction tissue which crossed the border from the normal zone into the infarct; it was part of the multidirectional network near the apex.

Figure 7

action potentials, a slower abatement of enhanced automaticity, and a persistence of prolonged action potentials.

To obtain an indication of whether the prolonged action potentials existed in the intact dog, we compared the responses of Purkinje cells within the infarcted zone and Purkinje cells within the normal zone of the intact heart to premature stimulation or rapid pacing. The spontaneous, rapid, irregular ventricular activity made precise measurements of refractory periods somewhat difficult. However, qualitatively the results were quite consistent. As the pacing rate increased, 2:1 block of Purkinje potentials was observed within the infarcted zone at a time when Purkinje potentials followed 1:1 at sites within the normal zone. Similarly, with a single premature stimulus Purkinje potentials recorded from the infarcted zone blocked at coupling intervals which still allowed a full response of Purkinje potentials within the normal zone. Typical examples are shown in Figure 8. Qualitatively similar results were obtained in eight other comparisons of infarcted and normal sites.

The initial depression of Purkinje cells within the infarcted zone may have been related in part to the trauma of excision and isolation. Although cells within the normal zone were not similarly depressed in acceptable experiments, it is possible that cells within the infarcted zone were selectively

Figure 8

Prolonged refractory periods of Purkinje cells within the infarcted zone in vivo. Standard lead aVF (first trace) and bipolar recordings from two endocardial sites in the infarcted zone (IZ endo) (traces 2 and 3) and one endocardial site in the normal zone (NZ endo) (trace 4) are shown. Endocardial recordings show Purkinje (P) spikes. A: Recordings during premature atrial stimulation (APC) while the basic driving rate was 100. B: Recordings during rapid drive: cycle length (CL) = 230 msec.
affected during excision and isolation because of a greater sensitivity to trauma. Purkinje cells within the infarcted zone appeared to be more sensitive to mechanical trauma than did Purkinje cells within the normal zone. Stroking the surface of the infarcted zone lightly with a metallic object such as the handle of a scalpel consistently produced relatively severe and lasting local depression of extracellular and intracellular potentials in comparison with the normal zone which was only slightly depressed and recovered quite rapidly after equivalent mechanical trauma. Because of this observation, care was taken to avoid direct contact with the endocardium of the infarcted zone during excision, dissection, and isolation of the preparation in the perfusion chamber. In most preparations the endocardial surface of the infarcted zone was not touched by instruments or fingers during these procedures.

The prolongation of phase 3 of the action potentials of the Purkinje cells in the infarcted zone would be expected to enhance the likelihood of reentry. It was very easy to produce repetitive activity with premature stimulation of the normal zone. Figure 9 illustrates a sampling of various patterns of activity initiated by a single premature stimulation of the normal zone coupled at 260 msec from the previous driving stimulus. Note that activation of the various sites in the preparation was quite asynchronous and, for the most part, continuous. Rapid pacing of the normal zone could produce irregular and multiple responses in incompletely polarized Purkinje cells within the infarcted zone, suggesting chaotic and fibrillatory spread of activation within the infarcted zone.

Manifest pacemaker potentials were never found within the normal zone, because the dominant pacemaker was always in the infarcted zone. Various forms of pacemaker activity were found within the infarcted zone. Soon after isolation, pacemaker cells were often hypopolarized (maximum diastolic potential of low amplitude) and manifested oscillatory patterns approximating sine waves. Later, after recovery, the firing rates were slower, the maximum diastolic potential greater, and the action potentials more typically formed. Figure 10 shows an example of a pacemaker potential recorded soon after isolation, whereas Figure 11 depicts a pacemaker potential from a more fully polarized cell. During the period of several minutes of recording from the cell of Figure 10, the amplitude of the transmembrane potential and its oscillations diminished. The pacemaker cell of Figure 11 showed periods of irregular discharge and unexpected long cycles. The irregular firing was not associated with shifts in the maximum diastolic potential or the threshold potential. It apparently was related to variation in the slope of phase 4 depolarization. The pacemaker cell in Figure 11 fired at a relatively slow rate and had a very long action potential—about 1000 msec. Entrance and exit block around pacemaker sites was common. Pacemakers in Figures 10 and 11 were parasystolic.
foci with both exit and entrance block during the period of recording.

Spontaneous firing of pacemakers within the infarcted zone was often followed by one or two closely coupled discharges. Recording from pacemaker fibers (Fig. 12) suggested that the pacemaker cell for the first discharge of the group did not initiate the subsequent coupled beats but was excited by a propagated impulse during those coupled beats. Spontaneous discharge of a pacemaker fiber was indicated if the transmembrane potential attained the level of the threshold potential before undergoing a transition to the rapid upstroke phase. Excitation from another cell was indicated if the rapid upstroke phase originated abruptly from a level of membrane potential other than the threshold potential, as illustrated by the second depolarization of site X in Figure 12A. Figure 12 shows intracellular and extracellular recordings from a pacemaker site and multiple other sites during a period of spontaneous rhythm characterized by grouped discharges. After the initial spontaneous firing initiated at site X, which was a pacemaker cell, all areas were excited except the region around site 3 close to the pacemaker site. Extracellular recordings in this vicinity detected no activity. Intracellular recordings showed depolarized quiescent cells or depolarized cells with very small action potentials. This area was an irregular band a few millimeters wide extending from the pacemaker site to the border of the infarcted zone. The initial set of discharges (I) was followed by a second set of discharges (II) of only the sites in the normal zone and at the border (2, 4, Z). It is probable that this second firing (II) of normal zone sites was initiated by an impulse which slowly moved through the area around site 3 after the pacemaker firing. At the second firing (II), the site at the border (Z) generated only a small action potential. It is likely that at some border areas the second firing was of sufficient amplitude and delay to excite cells in the interior of the infarcted zone with long refractory periods. This successful reexcitation of cells in the infarcted zone initiated the third set of firing (III). Note that the upstroke of a pacemaker cell (X) preceded the first (I) but not the second (II) or third (III) excitations of the normal zone.

Discussion

The methods used in this study generate more than the usual apprehensions about the introduction of experimental artifacts. The procedures of isolation and superfusion eliminated the special conditions that produced the infarcted zone, i.e., localized ischemia. After isolation, the identical conditions of superfusion for subendocardial cells of the infarcted and the normal zone might eventually obliterate distinctions between Purkinje cells in the two zones. It was possible to observe differences between infarcted and normal zone cells because the rate of disappearance of differences was relatively slow compared with the time required for isolation. However, there may have been other distinctive features of cells in the infarcted zone which rapidly disappeared during

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Spontaneously firing preparation showing grouped beating (trigeminy). Center: Schematic representation of the preparation shows the infarcted zone (stippled area), anterior papillary muscle (APM), and sites of intracellular (X, Y, and Z) and extracellular (1, 2, 3, and 4) recordings. A and B: Recorded (at different sweep speeds) within an interval of 10 minutes. In A the upstroke of the action potential of the pacemaker site (X) was used to trigger the oscilloscope sweep, so it does not appear on the sweep. After the initial firing (I) of all sites (except 3), sites in the normal and border zones (2, 4, and Z) were reactivated (II). After this second firing, all sites (except 3) were again reactivated (III). See text for discussion.

Another concern is the introduction of fallacious data by the experimental procedures. Purkinje cells within the infarcted zone were particularly sensitive to mechanical trauma, which produced relatively long-lasting diminution of resting and action potentials. Although contact with the endocardial surface was avoided, other unidentified factors in the excision, dissection, and isolation may have been injurious to unduly sensitive Purkinje cells in the infarcted zone. Because of these various possible sources of artifact in the data obtained in vitro, we looked in vivo for confirmatory findings. The recordings from the intact heart supported the inference that Purkinje potentials were depressed in vivo as well as in vitro, although perhaps less severely. The other distinctive features of isolated Purkinje cells in the infarcted zone were prolonged action potentials and enhanced automaticity marked by an increase in the rate of phase 4 depolarization. There is corroborative evidence that these prolonged action potentials and the enhanced automaticity are also present in the intact animal. Purkinje potentials recorded within the infarcted zone in vivo were consistently blocked at longer cycles than were Purkinje potentials recorded in the normal zone. Enhanced automaticity seen in the isolated Purkinje cells probably has a counterpart in the rapid multiformal ectopic firing observed in the intact dog during vagal-induced atrial arrest.

The characterization of the cell type surviving in the infarcted zone requires some scrutiny. The evidence indicated that only one cell population had survived in the infarcted zone. In contrast to the recordings from the normal zone, which showed composite electrograms containing rapid and slower spikes, the electrograms from the infarcted zone always consisted of single spikes. Also, all the cells in the infarcted zone, when recovery had occurred, had similar characteristics on intracellular recording. Classification of this surviving population of cells as Purkinje fibers is based on various electrophysiological characteristics. In the infarcted zone, the electrograms showed rapid spikes (1-3 msec in duration) like the spikes generated by the Purkinje fibers in the normal zone and unlike those generated by myocardial cells in the normal zone (6-10 msec). In addition, all the cells in the infarcted zone showed diastolic depolarization, a property of normal Purkinje fibers but not of normal myocardial cells. The configuration of the action potentials recorded in the infarcted zone, although prolonged, resembled that conventionally associated with normal Purkinje fibers. Vmax of the
logical features as ischemia, (2) normal Purkinje hypoxia does not produce the same electrophysiological potentials characteristic of Purkinje cells in the infarcted zone. From these pale strands of tissue in the normal zone, transmembrane potentials characteristic of Purkinje fibers were always recorded. It was possible to trace individual strands into the infarcted zone with intracellular and extracellular recordings and to demonstrate the electrophysiological characteristics of the infarcted zone in the same strand which showed only normal Purkinje fibers in the normal zone.

Since the studies of Trautwein and co-workers (10), it has been recognized that hypoxia over a period of hours results in loss of amplitude of resting and action potentials and enhanced automaticity of isolated Purkinje cells. However, in those studies, simple hypoxia produced shortening of the action potentials in contrast to the marked prolongation occurring after coronary occlusion. This discrepancy between the effects of simple hypoxia and ischemia on the duration of the action potentials of Purkinje fibers indicates that hypoxia is not the sole factor directly affecting Purkinje cells after coronary occlusion. Further evidence that factors besides lack of oxygen or glucose affected Purkinje fibers was provided by the observation that the diminished resting and action potentials and the enhanced automaticity returned toward normal in vitro during superfusion with hypoxic solutions lacking glucose. It is questionable that the supply of oxygen or foodstuffs is insufficient for Purkinje cells in the infarcted zone in vivo. It is a commonplace experience in the experimental laboratory that subendocardial Purkinje fibers can be maintained in a relatively normal electrophysiological state for many hours, even days, under conditions of superfusion with conventional Tyrode's solution. Left ventricular chamber blood should sustain subendocardial Purkinje cells in infarcted zones about as well as Tyrode's solution. We employed a solution equilibrated with 20% O2 so that the Po2 approximated the Po2 of the chamber blood, and we were able to sustain Purkinje cells for 24 hours without the appearance of the prolonged action potentials characteristic of Purkinje cells in the infarcted zone.

To recapitulate, there have been three findings suggesting that factors deleterious to Purkinje cells are generated in ischemic zones: (1) simple hypoxia does not produce the same electrophysiological features as ischemia, (2) normal Purkinje cells superfused for at least 24 hours with a solution similar to the chamber blood in content of dextrose and oxygen do not acquire all the features of cells in the infarcted zone, and (3) Purkinje cells altered by ischemia recover toward normal during superfusion with solutions poor in O2 and dextrose. The last finding requires more comment. If a deleterious factor is washed out during superfusion in vitro, why is it not removed just as efficiently by the left ventricular chamber blood? It is necessary to postulate that the deleterious factor is either present in blood or potentiated by blood. Another interpretation of the third finding is suggested by the observation that Purkinje cells in the infarcted zone are especially sensitive to mechanical trauma, undergoing prolonged depression after mild surface contact. Therefore, it is possible that the recovery in vitro represents the release of trauma-sensitive cells from the pulsatile pressure within the left ventricle. The different rates of normalization in vitro of the various ischemic features suggest an interplay of multiple factors released in the infarcted zone. It is unlikely that catecholamines are the major factor inducing the enhanced automaticity, since beta-receptor blockade did not much affect this phenomenon.

For many years, there has been a recurrent suspicion that factors other than hypoxia alone might be instrumental in the production of arrhythmias after coronary occlusion (11–20). To properly evaluate the large store of data relevant to this problem, it is necessary to sort out those studies concerned with the initial ventricular arrhythmias which occur within the first 30 minutes of coronary occlusion from those studies concerned with the delayed ventricular arrhythmias which occur more than 6 hours after occlusion. Harris (21) has suggested that the two phases of arrhythmias have different underlying electrophysiological mechanisms. A number of reports have proposed that the early arrhythmias are due to reentry in slowly conducting ischemic myocardium (5–8). Data implicating factors other than hypoxia in the genesis of arrhythmias have been gathered mainly in studies concerned with the first phase (16, 17, 22–25). The phase of delayed ventricular arrhythmias has not occupied as much attention with respect to this particular issue. One study (26) showed that systemic hypoxia mitigated the ectopic ventricular activity of the later phase.

We should consider whether these findings are relevant to myocardial infarction in man, that is, whether the animal model is appropriate. Ordi-
narily, patients with myocardial infarction do not display ventricular activity as prolific and multiformal as that shown by dogs on the first day after abrupt occlusion of the anterior descending coronary artery. In dogs, sinus slowing will uniformly unmask rapid and multifocal ventricular activity, if it has been obscured by sinus tachycardia (4, 27). In contrast, many patients with sinus bradycardia or heart block after coronary occlusion do not manifest enhanced ventricular pacemakers (28). However, these differences may be mainly in degree. Recently, accelerated ventricular rhythm in man has been recognized more frequently (in as many as 30% of patients who were continuously monitored) (29–31). Some workers have called attention to the resemblance between this arrhythmia in man and that occurring in dogs on the first day after occlusion. Like the canine phenomenon, accelerated ventricular rhythm in man leads to ventricular fibrillation comparatively rarely, and it is unmasked by sinus slowing. Our findings suggest that the cellular basis of this arrhythmia is enhanced automaticity of Purkinje fibers in the infarcted zone.

Our studies showed that Purkinje cells within the infarcted zone were prone to initiate repetitive reentrant activity because of the prolongation of phase 3 of repolarization. This feature may be important in man in the genesis of “malignant” paroxysmal ventricular tachycardia and ventricular fibrillation initiated by closely coupled ventricular extrasystoles (32, 33).

The disparity between the duration of action potentials of Purkinje cells in the infarcted zone and action potentials of cells in the normal zone conceivably might be a mechanism by which closely coupled extrasystoles could be generated. At the border of the infarcted zone, fully recovered Purkinje or muscle cells might be reexcited by current flow from contiguous Purkinje fibers in the infarcted zone which remain at a higher membrane potential during the prolonged repolarization phase. However, we did not document examples of reexcitation by this mechanism.

Finally, if the main bundle branches were affected by infarction, the very prolonged action potentials and the decreased conduction velocity might account for transient rate-dependent bundle branch block occurring in the course of clinical myocardial infarction. Thus, the Purkinje fibers in the infarcted zone have characteristics which might account for various rhythm and conduction disturbances observed in myocardial infarction in man.

References

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