Purkinje involvement in arrhythmias after coronary artery reperfusion

DAVID O. ARNAR AND JAMES B. MARTINS
Division of Cardiovascular Diseases, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City; and Veterans Administration Medical Center, Iowa City, Iowa 52242

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Arnar, David O., and James B. Martins. Purkinje involvement in arrhythmias after coronary artery reperfusion. Am J Physiol Heart Circ Physiol 282: H1189–H1196, 2002; 10.1152/ajpheart.00227.2001.—Previous studies have indicated that the endocardium may be responsible for a large portion of ventricular tachycardia (VT) seen with reperfusion of ischemic myocardium. To evaluate the role of the Purkinje system in nonreentrant VT arising from the endocardium after reperfusion, the anterior descending coronary artery was occluded for 20 min and then reperfused in 23 dogs after instrumentation of the risk zone with 21 multipolar plunge needles. VT with focal Purkinje origin was defined as a focal endocardial VT with Purkinje potentials recorded before the earliest endocardial myopotential. A total of 19 VTs (mean cycle length 214 ± 2 ms) were observed with 11 (58%) having focal Purkinje origin. Fifty-eight percent of the VTs degenerated to ventricular fibrillation, with occurrences of two or more independent foci per complex (seen in 7 of 11 compared with 1 of 8 nonsustained VTs). In conclusion, these data show that Purkinje tissue may be important in the genesis of reperfusion VT.

METHODS
Twenty-three healthy adult mongrel dogs were used for this study. The protocol was approved by the University of Iowa Animal Use and Care committee and conforms to the position of the American Physiological Society on research animal use.

Surgical preparation. Anesthesia was induced with the use of thiopental sodium (500 mg) and α-chloralose (100–200 mg/kg iv) as a bolus and maintained with a continuous intravenous infusion of α-chloralose dissolved in polyethylene glycol at 8 mg·kg⁻¹·h⁻¹. The animals were intubated and ventilated on a ventilator (Harvard Apparatus) with settings adjusted to achieve a physiological arterial PCO₂ (25–35 Torr) and to maintain normal Po₂ (80–150 Torr). An infusion of NaHCO₃ was used to maintain the pH within physiological range (7.30–7.45) if necessary. Serum electrolytes K⁺ (3.6–5.0 meq/l), Mg²⁺ (1.5–3.0 mg/dl), and Ca²⁺ (8.5–10.5 mg/dl) were intermittently measured. Arterial pressure was continuously monitored via a femoral arterial line and the femoral vein was cannulated for infusion of drugs and saline.

The heart was accessed through a median sternotomy and a snare was used to occlude the left anterior descending (LAD) coronary artery immediately distal to the first septal perforator. After the experiment, the dogs were euthanized by induction of VF.

Electrophysiological measurements. A bipolar electrode was used to pace the right atrium at two times diastolic threshold with pulses of 2-ms duration at a cycle length (CL) of 300 ms. To control the heart rate, atrial pacing at this CL and sinus node clamping was performed. While surface electrocardiographic leads II and V₅R were continuously monitored, all six limb leads (I, II, III, aVR, aVL, and aVF) and lead V₅R were recorded. Before coronary artery occlusion, 21 multipolar plunge needles were inserted into and surrounding the risk zone of the LAD coronary artery occlusion as

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previously described in detail (1). Needles were placed in five rows of one to six electrodes. The first row was placed diagonally into the septum along the right side of the anterior descending artery and the last row at the end of the first regional branch (Figs. 2 and 3). Each needle recorded six bipolar electrograms from circumferential electrodes made from Teflon-insulated tungsten wire was 1 mm apart, enabling recordings from 126 sites. Spacing between the needles was ~10 mm (1).

Electrograms were recorded simultaneously on two separate computers, one for the three endocardial-most bipoles and the other for the three epicardial-most bipoles (1). Signals from the three endocardial-most electrodes were amplified by a gain of 100, band-pass filtered between 3 and 1,300 Hz, and sampled at 3.2 kHz. The epicardial electrograms were sampled at frequency of 1 kHz per channel and band-pass filtered at 30–300 Hz. Three-dimensional activation maps were constructed from multiplexed signals. Data from both acquisition systems were incorporated for the construction of three-dimensional activation maps with a common surface electrocardiogram (lead II) recording atrial pacing spikes allowing for alignment of signals from both computers.

Each needle had 16 unipoles that were used to select the 6 optimal bipolar electrograms, which were adjusted to maximize the capability to record Purkinje signals on the endocardial-most bipolar. The adjustment was performed with the use of sequential recordings on a storage oscilloscope for each bipolar. A switching box was utilized to connect the selected bipoles to each amplifier. The length of the needles (22 mm with circumferential electrodes covering the proximal 16 mm of the needle shaft) traversed through the left ventricular wall into the left ventricular cavity. The epicardial-most bipolar recorded an electrogram from the epicardium, and subsequent bipoles recorded electrograms sequentially through the myocardial wall. The endocardial-most bipolar was used to record Purkinje potentials when they could be identified. Purkinje potentials were defined as high-frequency, low-amplitude spikes preceding local muscle activity, as others have previously described (14, 15) and further identified by adhering to previously published criteria (1,2) from this laboratory, including 0.5-mV spikes lasting 1–2 ms, preceded by the larger and longer muscle spike (1–11 ms) and the surface QRS on the intramyocardial electrogram. If a Purkinje potential was not identified for a given electrode, no activation time was marked for the endocardial most electrogram. Purkinje signals were generally stable on a beat-to-beat basis. To be considered mechanistically involved in the observed VT, signals had to be consistently observed over the study period, as indicated below. If Purkinje signals were seen on more than one bipolar, the best signal was chosen for a given plunge needle. Activation maps were constructed as described before (1).

Definitions. VT was defined as at least three or more premature ventricular complexes in sequence. VT was considered sustained after >30 s or because either cardioversion or pace termination was required before 30 s due to hypotension. VT lasting <30 s and spontaneously terminating before that time was considered nonsustained. If VT degenerated to VF, it was classified as such.

VT was designated to have a focal origin when no electrical activity could be recorded on all adjacent sites in three dimensions between the latest activation of one QRS complex and the earliest of the next QRS. Moreover, conduction from the site of earliest activity to adjacent electrodes could not manifest conduction delay, which might account for a majority of the CL of the VT. This definition of focal VT does not fully exclude the possibility of microreentry as a mechanism, although reentry of large circuits is unlikely.

Purkinje origin of VT was defined as a focal endocardial mechanism with recording of a Purkinje potential before the QRS on the lead recording the earliest activity. Thus the earliest activity of the mapped sites is in the Purkinje fibers. Purkinje potentials had to be identified on electrograms during atrial pacing before and after coronary occlusion and during VT to be considered mechanistically involved.

Mechanisms were defined as reentrant when the earliest activation site was located immediately adjacent to the site of the latest activation from the previous complex and continuous diastolic activation was recorded between complexes. Reentrant mechanisms also demonstrated initial unidirectional and functional block to the subsequent earliest site of activation. VF was defined as rapid, irregular, and polymorphic electrical activity of varying amplitude developing after the onset of reperfusion VT.

Ischemia was defined as a reduction in voltage of electrograms as described by Ruffy et al. (13). In addition, we used changes in transmural activation times and macroscopic evidence of cyanosis and hyperemia as indicators of ischemia–reperfusion.

Experimental protocol. After instrumentation of the myocardium with the multipolar plunge electrodes, dogs were observed for spontaneous VT for ~45–60 min before coronary artery occlusion to exclude VT that might occur from mechanical artifact due to insertion of the needles. The LAD was then occluded with a snare and an occluder, which allowed for easy restoration of flow in the vessel after 20 min. All spontaneous VTs occurring in the first 20 min after occlusion were recorded and stored on computer. After 20 min, the occluder was released and all spontaneous VT occurring in the first 2 min after occlusion was recorded and stored. Restoration of blood flow was assessed by both visual reactive hyperemia of the risk zone and changes in the transmural activation times.

Statistics. Data are expressed as means ± SE. Student’s t-test was used for comparison between groups. A value of P < 0.05 was considered significant.

RESULTS

Of the 23 dogs studied, VT occurred within 1 min of reperfusion in 19 (83%). Of these 19 VTs, 11 (58%) degenerated to VF usually within seconds whereas 8 were nonsustained. All dogs had visual reactive hyperemia of the ischemic risk zone on reperfusion. The mean number of multipolar needles recording Purkinje signals at the beginning of each experiment was 9 ± 0.7.

Transmural activation times. Before coronary artery occlusion, the mean transmural activation time was 37 ± 1 ms. After 20 min of coronary artery occlusion, the transmural activation time had increased to 62 ± 8 ms (P < 0.05 vs. baseline), consistent with conduction delay caused by ischemia. Immediately after reperfusion, the activation times were 54 ± 5 ms (P = not significant vs. 20 min of occlusion), but at 5 min after reperfusion, the transmural activation times had decreased to 41 ± 2 ms (P < 0.05 vs. early reperfusion). These conduction data support myocardial ischemia that occurs during occlusion and subsequent restoration of blood flow to the myocardium during the reperfusion period.
Mechanism and sites of origin of reperfusion VT. The mean CL of the reperfusion VT was 214 ± 2 ms. The 11 VTs that degenerated to VF had a CL of 194 ± 3 ms compared with a CL of 236 ± 3 in the 8 nonsustained VTs (P < 0.05).

Of the VTs seen, 11 (58%) were of focal Purkinje origin, whereas 2 (11%) were of focal endocardial or subendocardial origin. The other six had focal epicardial or focal midwall origins (Table 1). Figure 1 shows surface and intramural electrograms of a VT of focal Purkinje origin. The last atrial paced complex and the first three complexes of reperfusion VT are shown and Purkinje signals precede the earliest myocardial activity during the VT complexes. Figure 2 shows an activation map of the first VT complex as an example of a focal Purkinje VT. The earliest activity was seen in the Purkinje layer (−17) and the earliest adjacent muscle activation was −2 ms; the Purkinje muscle activation time (15 ms) was longer than during atrial pacing (4.8 ± 1.8 ms), which is consistent with Purkinje origin of VT. Absence of extraordinary (>50% of CL of VT) conduction delay in the surrounding electrodes makes reentry unlikely. There is evidence of conduction delay in the remote epicardium although this activity did not reenter to the Purkinje layer (see Fig. 3).

Maintenance of VTs. The majority of VTs seen after reperfusion were also maintained by complexes of focal origin. Macroreentry was rare during maintenance complexes of the VTs (Table 2). However, conduction delay was frequently seen in the epicardium-creating conditions, such as unidirectional block, which might promote reentry (Fig. 4). The VTs that subsequently degenerated to VF were in 7 of 11 cases maintained by complexes where more than one independent site of early focal activity was seen (Table 2), examples of which are shown in Figs. 3 and 4. In Fig. 3, the earliest focus of activity is in the Purkinje layer (−31 ms). However, there is also early activity in the epicardial layer (−10 ms) that appears to be independent of the activity in the Purkinje layer; the transmural conduction being too slow to result in the early epicardial activation time seen. In Fig. 4, potentially three separate sites of early activity, including focal Purkinje, focal subendocardial, and subepicardial reentry are observed in a late complex before occurrence of VF. These are examples of the multiple sites of independent early electrical activity, which could facilitate degeneration to VF. Complexes with more than one site of early activity were seen only on a single occasion.

Table 1. Sites of origin of focal VTs occurring immediately after reperfusion of ischemic myocardium

<table>
<thead>
<tr>
<th>Activity sites</th>
<th>No. of VTs</th>
<th>%Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje</td>
<td>11</td>
<td>58</td>
</tr>
<tr>
<td>Epicardium</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Endocardium</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Numbers show sites of earliest activity of all VTs (n = 19) and %contribution of different sites. VT, ventricular tachycardia.
during maintenance of VTs, which were self-terminating and did not degenerate to VF.

Relationship to occlusion VT. Because it has been described that the presence of VT during reperfusion is more common if VT occurs during coronary occlusion, we also recorded all occlusion VT during the first 20 min of the experiment with the purpose to compare reperfusion and occlusion VT. Eight of the 19 dogs (42%) that had reperfusion VT also had VT occurring during the 20-min period of coronary artery occlusion. The mean CL of the occlusion VTs was 251 ± 1 ms ($P$ = not significant vs. CL of reperfusion VT) and all the VTs were nonsustained (3–7 complexes). As we previously reported (1), the occlusion VTs frequently took origin from Purkinje, although not the same Purkinje sites as with reperfusion. The 4 dogs of the total 23 studied without reperfusion VT also had no VT during the occlusion phase.

DISCUSSION

The main findings of this study are twofold. First, these data imply that the Purkinje system might be importantly involved in the genesis of reperfusion VT. The results presented herein are consistent with previous data suggesting that focal mechanisms arising in the subendocardial area may be a common source of early reperfusion VT. However, a prior study did not differentiate between subendocardial myocardium and Purkinje tissue immediately overlying the endocardium. Second, we observed that subsidiary sites of VT origin per complex of VT were associated with degeneration to VF. These results may further add to our understanding of the origin of early reperfusion arrhythmias.

Reperfusion VT. The occurrence of VT and VF after reperfusion of ischemic myocardium is somewhat time dependent, that is, the peak incidence of arrhythmias...
occurs after ~20–30 min of coronary artery occlusion and decreases thereafter. In this study, the incidence of reperfusion VT after 20 min of ischemia was 75% and the VTs commonly had a focal Purkinje origin. Previously, Janse et al. (6) have shown that, whereas complete and incomplete reentrant waveforms were seen during transition of reperfusion VT to VF, no evidence of reentry was seen during the initiation of VT in isolated canine and porcine hearts. Ideker et al. (5) suggested that during transition of reperfusion VT to VF the complexes were initiated by a focal source in the ischemic border zone in the dog. These studies were somewhat limited in that the number of recording sites did not allow for exact delineation of the mechanism of the arrhythmia. In a more recent study, Pogwizd and Corr (11), using a detailed mapping system with 232 recording sites in the feline heart, demonstrated that nonreentrant mechanisms involving the endocardium were the source of 75% of all reperfusion VT seen. The data presented in our study, showing a preponderance of focal Purkinje VT after reperfusion, are not in conflict with the results published by Pogwizd and Corr. Their mapping system, although with higher spatial resolution in the cat model than the one used in the present dog model, did not record Purkinje potentials.

Table 2. Sites of earliest activity of maintenance complexes of all 19 reperfusion VTs prior to spontaneous termination or degeneration to VF and % contribution of different sites

<table>
<thead>
<tr>
<th>Focal activity sites</th>
<th>No. of Complexes</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje</td>
<td>62</td>
<td>48</td>
</tr>
<tr>
<td>Endocardium</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Midwall</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Epicardium</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Reentry</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Multiple sites</td>
<td>18*</td>
<td>14*</td>
</tr>
</tbody>
</table>

*Two or more independent sites of early focal activity during the same complex.

Fig. 3. Activation map of second VT complex of electrogram shown in Fig. 1. The earliest focus of activity (~31 ms) is again seen in the Purkinje layer with an appearance of a focal origin. Early activation (~10) is also seen in the epicardium and this activity is earlier than expected from transmural activation, suggesting a secondary independent focus of early activity. Immediately next to the early epicardial site is the conduction block and the development of conduction delay around the site of block. Color code is the same as in Fig. 2.
and was therefore not able to distinguish between Purkinje tissue and muscle as the source of focal VT originating from the endocardium. The activation maps of the VTs originating from Purkinje tissue in this study showed that the earliest focus of activity was in the Purkinje layer. There was rapid activation of surrounding Purkinje tissue before activation of overlying muscle, which was later than that observed with atrial pacing. This is consistent with a focal origin of VT within the Purkinje layer. We did observe evidence of slow conduction and even conduction block in the epicardium, but this activity was more often than not significantly later than the focus of origin. Our data may therefore complement the findings of Pogwizd and Corr by further characterizing the contribution of the Purkinje system and myocardium to VT of focal endocardial origin. Although reentry has been described as causing reperfusion VT, such was not seen during this study.

**Possible mechanisms of reperfusion VT.** Kaplinsky et al. (7) have described that reperfusion VT tends to occur in two distinct phases: immediate and delayed. It has also been proposed that different mechanisms may contribute to these two phases of arrhythmias. In this study, we studied only the immediate phase reperfusion arrhythmias. The early phase correlates with fragmentation of local electrograms and continuous diastolic activity, which suggests that conditions for reentry are present. Evidence from mapping studies has, however, suggested that nonreentrant mechanisms may be important in the genesis of reperfusion VT. In this study, evidence of conduction delay and perhaps incomplete reentrant waveforms were seen in the epicardium, although in most cases, the VT complex had been initiated by a focal source in the Purkinje or endocardium.

The underlying mechanisms of VTs with a focal origin in this study could not be ascertained. We speculate, however, that macroreentry is unlikely given absence of significant conduction delay and block in the areas surrounding the electrodes showing the earliest focus of activity. Microreentry and reflection involving
a small area of adjacent myocytes are possible mechanisms and this has been shown to occur in very small volumes of tissue (12). Triggered activity, involving either delayed afterdepolarizations or early afterdepolarizations (EADs), has previously been implicated as a possible mechanism of reperfusion VT with focal origin (11). Triggered activity has also been observed during in vitro studies using simulated ischemia-reperfusion (9). In this study, however, EADs would seem to be an unlikely etiological factor because the atra were paced at a CL of 300 ms and the occurrence of EADs is somewhat dependent on slower heart rates. Although, as previously discussed, abnormal automaticity may contribute to delayed reperfusion arrhythmias, there does not appear to be data implying automaticity as a cause of immediate reperfusion VT (18).

Occurrence of VF. VF was seen in 58% of all cases where reperfusion VT occurred. VF occurs more commonly with reperfusion than coronary occlusion (16). In Pogwizd and Corr’s (11) study on reperfusion arrhythmias, VF occurred in 33% of animals studied. The CL of the VTs, which subsequently degenerated to VF in this study, had a shorter CL than those who were self-terminating. This is in part due to acceleration of the VT CL seen just before degeneration to VF. In 7 of 11 cases where VTs degenerated to VF, more than one independent site of early activity was noted during one or more of the maintenance beats, whereas this was seen in only one of eight of the nonsustained VTs. The observation of more than one independent site of early activity immediately after reperfusion likely reflects the disorganized recovery of myocardial tissue from ischemia. The recovery of ischemic tissue during reperfusion is nonhomogeneous as evidenced by areas of slow conduction in this study, especially in the epicardium. The preponderance of multiple independent sites of early activity among those animals where VT degenerated to VF suggests that the relationship is causal.

Limitations. The recording of electrical activity was limited to the risk zone of the proximal LAD coronary artery occlusion and areas immediately surrounding the risk zone. This does not exclude that some of the activity seen could have originated from remote areas, although such remote activity could hardly be related to ischemia or reperfusion. However, in the reperfusion VT in this study, pre-QRS activity was fully surrounded by electrodes in the ischemic and border areas in all instances, strongly suggesting that the focus of origin was within the anticipated risk zone from the LAD occlusion.

Also, as previously mentioned, the spatial resolution of the mapping system used does not allow for exact delineation of the mechanisms underlying VT of focal origin. In addition, it may be possible that if microentry was a mechanism, some of the VTs may have involved endocardial muscle as a part of that microreentrant circuit.

Another potential limitation arises from the insertion of transmural needles, which could effect the occurrence of arrhythmias, both before and during occlusion as well as with reperfusion. Although we did not compare the occurrence of reperfusion arrhythmias in dogs with and without transmural intramyocardial needles, we carefully observed each animal for 45–60 min after insertion of the needles for spontaneous VT. No spontaneous arrhythmias were seen during this period.

In conclusion, the results of this study implicate the Purkinje system as a potentially important component in the development of VT occurring immediately after reperfusion of ischemic myocardium. These results are both in accordance with and may complement previous data, which show that nonreentrant mechanisms arising in the endocardium are a predominant source of reperfusion VT.

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