Repeated 15-Minute Coronary Occlusions in Pigs Increase Occlusion Arrhythmias but Decrease Reperfusion Arrhythmias That Are Associated With Extracellular Hypokalemia

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Objectives. We sought to evaluate the effects of repetitive 15-min coronary occlusions followed by 45-min reperusions on the incidence of occlusion and reperfusion arrhythmias in pigs.

Background. Brief 2- to 5-min coronary occlusions seem to exert a protective effect on occlusion and reperfusion arrhythmias. However, because clinical ischemic episodes are often longer, it would be appropriate to assess whether such protection also occurs when longer cycles of occlusion–reperfusion are produced.

Methods. Three to four cycles of 15-min coronary occlusions with 45-min reperusions were performed in 34 pigs, and changes in ST segment and incidence of ventricular arrhythmias were assessed. Plasma potassium ion concentrations in eight pigs and blood gas in six were measured from blood from the ischemic area during reperfusion.

Results. Repetitive occlusions were associated with a progressively higher ST segment elevation and a higher incidence of ST segment alternans (p < 0.001) and ventricular fibrillation (VF) (p < 0.01). However, during repetitive reperusions, normalization of the ST segment was increasingly faster, the incidence of VF was progressively reduced (p < 0.03), and there was progressively less severe regional acidosis ([mean ± SD] 7.06 ± 0.12 vs. 7.26 ± 0.06, p < 0.05) and hypokalemia (1.9 ± 0.7 vs. 2.3 ± 0.4 mEq/liter, p = NS).

Conclusions. The progressive electrocardiographic deterioration and increasing incidence of ventricular arrhythmias during repetitive 15-min occlusions in pigs suggest increasing metabolic derangement. However, the progressively faster normalization of the ST segment and the reduced incidence of ventricular arrhythmias during reperfusion suggest an increasingly faster restoration of the metabolic and ionic balance.

Methods

Forty pigs weighing 15 to 20 kg were anesthetized with metomidate, a short-acting, nonbarbiturate hypnotic (4 mg/kg body weight intravenously), and sodium pentobarbital (15 to 20 mg/kg intravenously). Under mechanical ventilation with a Bird Mark 8 respirator, a midsternal incision was performed, and the heart was suspended in a pericardial cradle. A 16-gauge cannula was introduced through the right carotid artery for pressure monitoring and blood gases sampling to ensure adequate ventilation. A bipolar 5F pacing wire was sutured to the right atrial appendage and connected to a demand pacemaker to maintain a constant heart rate (90 to 100 beats/min). The anterior descending branch of the left coronary artery was dissected below the first diagonal branch and isolated with a 5.0 Prolene snare. To obtain epicardial and endocardial direct current electrograms, intramural nonpolarizable electrodes were inserted. Each electrode consisted of two polyethylene tubes 10 cm long and 0.6 mm in diameter containing a fine cotton thread filled with isotonic saline. One tube served as an epicardial electrode and the other as a subendocardial electrode. The distal part of the epicardial tube was glued 3 cm from the end of the endocardial tube. The endocardial tube was inserted by puncture into the ventricular myocardium and advanced until the cotton wick of the epicard-
dial tube contacted the epicardium. At this position, the small hole previously made on the endocardial tube 7 mm below the epicardial recording site allowed the cotton wick to contact the subendocardial extracellular space. To avoid expulsion of the electrode by ventricular contractions, the electrodes were carefully sutured to the adjacent subepicardial layer by a lightly tied Mersiline 5/0 suture. The proximal ends of both tubes were inserted into a saline-filled hole carved in a Perspex plate. The potentials in each saline-filled hole were sequentially recorded with a pair of hand-held cotton wick electrodes connected by a silver chloride wire to a high input impedance buffer amplifier. The differential amplifier measured the potential differences between the saline-filled holes and the relative zero potential obtained from another wick electrode attached to the mediastinal fat. Two to four electrodes were placed in the ischemic area and another one or two in the nonischemic area, at least 1 cm outside the border zone.

In a group of 11 animals, a 6F catheter was introduced through the right internal jugular vein and advanced through the coronary sinus into the great cardiac vein until 2 cm below the level corresponding to the site of occlusion of the left anterior descending branch, for blood sampling from the ischemic area. Sodium and potassium ion concentrations measured in eight pigs and blood gases also measured in the last six, were compared with samples drawn simultaneously from the carotid artery line. Arterial pressure was measured through a 128OC Hewlett-Packard pressure transducer and was continuously recorded on an Elema Mingograph ink jet recorder. Epicardial and endocardial electrograms were simultaneously recorded on an Elema Mingograph ink jet recorder.

Protocol. After control recordings were obtained, an occlusion of the left anterior descending coronary artery branch was performed using an atrumatic arterial clamp. In 40 pigs the clamp was released after 15 min of occlusion and a 45-min reperfusion period was allowed. Thereafter, two more identical cycles of occlusion-reperfusion were performed, and a fourth cycle was performed in the last nine experiments. Three animals had to be excluded because of poor electrocardiographic (ECG) recordings and three others because of repetitive defibrillations in one of the cycles. Thus, 34 animals were available for analysis of three cycles and 9 for four cycles. In the 11 pigs in which a catheter had been introduced into the great cardiac vein, 2-ml blood samples for K+ and Na+ concentrations were drawn before occlusion and at 2, 5, 10, 15 and 30 min of reperfusion during the first three cycles. Because three of these pigs were excluded for repeated defibrillations, eight remained available for ionic analysis. At the same intervals and in the last six of these eight pigs, 1-ml samples for blood gas and acid-base variables (partial pressure of oxygen [PO2]; partial pressure of carbon dioxide [PCO2]; pH and base excess) were also drawn from the great cardiac vein and from the arterial line.

Epicardial and endocardial electrograms were continuously recorded from one of the electrodes of the ischemic area. In addition, and at 5-min intervals, recordings from all electrodes were taken at 100-mm/s paper speed and at a sensitivity of 1 mV/mm. The ST segment measured was the addition of the TQ segment depression, measured from the zero potential level to the straight part of the PQ interval, and the ST segment, also from the zero potential level. The latter was evaluated at a constant interval of 150 ms from the beginning of the q wave. Episodes of ventricular tachycardia (runs of more than 3 consecutive premature beats) and ventricular fibrillation (VF) were analyzed. Arterial pressure was also continuously recorded.

Statistical analysis. The data were analyzed with the use of the BMDP statistical package (Statistical Software Inc.). The Wilks test was used to assess whether distribution of the variables was normal or abnormal. The Student t test was used for comparison of two mean values with normal distribution and the Mann Whitney U test for those with abnormal distribution. Analysis of variance was used for comparison of more than two mean values or more than two repeated measurements with normal distribution and the nonparametric Kruskall-Wallis test for mean values with abnormal distribution. Multivariate chi-square tests were used to compare categoric variables. Results are expressed as mean value ± SD, and p < 0.05 was considered significant.

Results

The epicardial ST segment at 15 min of occlusion was progressively higher in the four different cycles (p < 0.02 to p < 0.002) (Fig. 1), and endocardial ST segment changes followed a comparable trend. The incidence of ST segment alternans increased progressively from the first to the fourth coronary occlusions (p < 0.001) (Fig. 2). During reperfusion, the epicardial and endocardial ST segments returned progressively faster toward the isoelectric line in the subsequent reperusions; the difference between the first and the fourth was particularly remarkable within the first 5 min (p < 0.01) (Fig. 1). Moreover, during the first reperfusion, a consistent but transient reelevation of the ST segment was observed in each experiment.

Occlusion arrhythmias. The incidence of ventricular tachycardia was low but tended to be progressively higher, whereas the incidence of VF was progressively higher from the first to the fourth occlusion (Fig. 3). Ventricular tachycardia and VF usually developed between minutes 10 and 15, and of 27 episodes of VF, 19 (70.4%) were preceded by ST segment
Figure 1. ST segment changes during four 15-min coronary occlusions (I to IV), followed by 45-min reperfusion (R). Maximal (Max) ST segment elevation was attained between minutes 10 and 15. During the first 5 min, changes in occlusions II, III and IV were less apparent, but beyond minutes 8 to 10, a reverse trend was observed. Differences were most remarkable between occlusions I and IV. During reperfusion, differences were even more striking, with a reelevation of the ST segment during the first minute of reperfusion IV is another salient feature.

Figure 2. Incidence of ST segment alternans gradually increased through the consecutive occlusions and tended to progressively occur at an earlier interval (I: 9.2 ± 2.2 min; II: 7.6 ± 2.6 min; III: 7.3 ± 2.6 min; IV: 6.9 ± 2.3 min). Differences between occlusions were significant (p < 0.001).

Alternans. Because the incidence of VF was lower than the incidence of ST segment alternans, the sensitivity of the latter to predict VF was low (31%). However, the specificity and predictive accuracy were high (88% and 70%, respectively).

Reperfusion arrhythmias. During reperfusion, the incidence of ventricular arrhythmias was progressively reduced, and the most striking difference was found between the first and the fourth reperusions (Fig. 4). All episodes of VF occurred within the first 60 s of reperfusion, and in the second (10 [83%] of 12), third (9 [90%] of 10) or fourth reperusions (1 [50%] of 2), VF had occurred during the first reperfusion in most experiments.

Ionic, acid–base and blood gas changes during reperfusion. With respect to preocclusion levels, there was a transient decrease in K+ concentrations at 2 min of reperfusion that tended to be lower during the first reperfusion than during the subsequent reperusions (Fig. 5). Sodium ion concentrations did not vary significantly during reperfusion.

In the six pigs in which acid–base and blood gas variables were analyzed, there was a decrease in pH in the first 2 min of reperfusion that resulted from a reduced base excess and from an increased Pco2, which was most severe in the first reperfusion (Tables 1 to 4, Fig. 6 and 7). The Po2 increased sharply during the first 2 min of each reperfusion, which is consistent...
with the postischemic hyperemic response. However, during the first reperfusion, PO, continued to increase significantly until the first 10 min and remained at a higher level than during subsequent reperfusions (Table 4, Fig. 7).

Discussion

Occlusion arrhythmias. The main findings of our study are the increasingly higher incidence of severe ventricular arrhythmias during occlusion and its progressively reduced incidence during reperfusion in pigs undergoing repetitive 15-min coronary occlusions with 45-min reperfusions. Previous studies have shown that severe ventricular arrhythmias develop within the first 30 min of a coronary occlusion (6–10) and that VF tends to occur between minutes 20 and 30 (7,9,10). Thus, the absence of VF during the first occlusion of 15 min is concordant with these observations. However, during subsequent occlusions, our results are at variance with the reduced incidence of VF seen in dogs (1, 3) or rats (3), using preconditioning with occlusions up to 5 min in duration. The increasing incidence of serious arrhythmias observed in our animals paralleled the progressive deterioration of the electrograms. Hence, ST segment elevation at 15 min was progressively higher in the consecutive occlusions and was preceded by an also increasingly higher incidence of ST segment alternans, as previously reported from our laboratory (11). This finding is of
Figure 5. Potassium ion (K⁺) concentrations in the great cardiac vein during reperfusion. With respect to preocclusion (Control) levels, initial reperfusion values were consistently reduced in the three cycles and gradually returned to preocclusion levels at 10 min. The increasingly higher preocclusion levels from occlusion I to III possibly reflect the cumulative extrusion of intracellular K⁺ during repetitive ischemia.

particular relevance in view of the recognized association between the latter and the development of VF (12–16). Therefore, it is conceivable that there was a progression in the severity of ischemia, perhaps because the 45 min of reperfusion were not enough to restore adequate metabolism. In addition, this cumulative effect might have resulted in an increasing concentration of norepinephrine, which seems proportional to the duration of ischemia in occlusions of 15 to 60 min in dogs (17) and may contribute to occlusion arrhythmias (18–20). Our results, moreover, are in line with those from Fleet et al. (21) who found, also in pigs, that during five coronary occlusions of 10 min followed by 50-min reperusions, the incidence of VF increased from 0% to 71%. Thus, the lack of protection of repetitive occlusions in our study, as opposed to the protection seen in previous reports (1–3), may be probably accounted for by the different duration of occlusions or reperusions. In fact, the latter were much shorter in the cited reports (1–3).

Reperfusion arrhythmias. Contrary to the increasing incidence of arrhythmias during consecutive occlusions, repetitive reperusions were associated with a progressive reduction in the occurrence of VF. Overall, incidence of reperfusion VF and ventricular tachycardia was significantly higher than during occlusion, in agreement with previous reports (7,22,23), at least in occlusions lasting from 5 to 30 min. It has been shown (1,2,4,5) that preconditioning with shorter occlusion periods reduces the incidence of reperfusion arrhythmias. Our study confirms that this protective effect is also apparent in pigs with repeated 15-min occlusions. Likewise, Osada et al. (5), using isolated rat hearts with three cycles of 15-min occlusions followed by 5-min of reperfusion, also evidenced a progres-

| Table 1. Changes in pH From Venous Drainage of Ischemic Zone During Repetitive 15-Minute Coronary Occlusions in Six Pigs |
|-----------------|-----------------|-----------------|
|                 | Occlusion I     | Occlusion II     | Occlusion III    |
| Control         | (mean ± SD)     | (mean ± SD)     | (mean ± SD)     |
| Venous          |                |                |
| Arterial        | 7.39 ± 0.10*   | 7.38 ± 0.10†   |
| Reperfusion (venous) |
| 2 min           | 7.06 ± 0.12*   | 7.25 ± 0.07†   |
| 5 min           | 7.30 ± 0.08*   | 7.35 ± 0.08*   |
| 10 min          | 7.38 ± 0.10*   | 7.38 ± 0.10*   |
| 15 min          | 7.40 ± 0.10*   | 7.37 ± 0.09*   |
| 30 min          | 7.38 ± 0.10*   | 7.37 ± 0.10*   |
|                | *(p < 0.01)†<sub>0.03</sub> |

| Table 2. Changes in Base Excess (mmol/liter) From Venous Drainage of Ischemic Zone During Repetitive 15-Minute Coronary Occlusions in Six Pigs |
|-----------------|-----------------|-----------------|
|                 | Occlusion I     | Occlusion II     | Occlusion III    |
| Control         | (mean ± SD)     | (mean ± SD)     | (mean ± SD)     |
| Venous          |                |                |
| Arterial        | 2.6 ± 3.0*     | 1.1 ± 2.2†     |
| Reperfusion (venous) |
| 2 min           | -8.9 ± 3.8*    | -2.8 ± 3.5†    |
| 5 min           | -1.4 ± 1.7     | -0.1 ± 2.0     |
| 10 min          | 1.7 ± 2.1      | 2.5 ± 2.3      |
| 15 min          | 1.5 ± 0.9      | 2.1 ± 1.4      |
| 30 min          | 1.4 ± 1.1      | 2.5 ± 1.6      |
| *(p < 0.01)     | *p < 0.03</sub> |
of coronary occlusion in dogs and then decreased progressively. Similarly, Camacho et al. (25) also documented that during the first 10 min of a coronary occlusion in pigs, there was a remarkable lowering of the pH in the ischemic area, but it remained unchanged thereafter. Moreover, Fleet et al. (21) showed that lowering extracellular pH was progressively less important through 5- to 10-min-consecutive occlusions with 50-min reperusions in pigs, and Warner et al. (26) reported comparable findings. Thus, it is likely that repetitive cycles of occlusion and reperfusion may deplete the metabolic substrate progressively, so that in each new cycle less catabolites are produced, although the extent of ischemia possibly increases. Alternatively, as suggested by Murry et al. (27) and Ricimer et al. (28), it may be that during repeated 5-min occlusions and 5-min reperusions, there might be a downregulation of anaerobic glycolysis with adenosine triphosphate (ATP) preservation (27). More recently, Kida et al. (29), using a similar model, found that four cycles led to reduced ATP consumption and to a lesser decrease in pH during a subsequent 20-min occlusion than a control occlusion. Accordingly, we observed that the po2 in the great cardiac vein before occlusion was higher in the second and third cycles than in the first, possibly suggesting lesser oxygen consumption. Indeed, some studies point to the reduced oxygen consumption in the stunned myocardium (30), which might explain the lesser consumption of ATP and production of acid catabolites.

**Potassium ion changes.** We encountered a noticeable decrease in K+ concentration in the great cardiac vein during the first minutes of reperfusion, which tended to be lowest in the first reperfusion. Although it is unlikely that this decrease would correspond to a systemic rather than a regional change, the lack of simultaneous K+ measurements in a systemic venous sample may hypothetically question our results. Our finding was unexpected because in line with the increased extracellular K+ demonstrated during ischemia (31,32), which at least in part may explain the ST segment elevation (33–35), we anticipated finding the highest K+ values at the onset of reperfusion and at a higher level than those at preocclusion levels. Fleet et al. (21) documented in pigs that repetitive 10-min coronary occlusions were associated with similar progressive increases in extracellular K+, except for the first occlusion. Because our occlusions lasted 15 min and the difference in maximal ST elevation was encountered between minutes 12 and 15, we cannot exclude that K+ could have further increased in this interval in the consecutive occlusions. This hypokalemia was not related to hemodilution because regional Na+ concentrations remained unchanged. Moreover, because the first samples were drawn at 2 min, it is conceivable that earlier samples could have shown even more remarkable changes, but not necessarily. Thus, because severe arrhythmias already occurred within the first 60 s, we cannot be certain that they were indeed associated with hypokalemia. However, along with our findings, Tosaki et al. (36) reported in isolated guinea pig hearts that during coronary occlusions of 15 to 25 min, there was a decrease in the myocardial concentration of K+ in dry tissue from 260 mmol/liter per kg to 210. However, during

### Table 3. Changes in Partial Pressure of Carbon Dioxide (mm Hg) From Venous Drainage of Ischemic Zone During Repetitive 15-Minute Coronary Occlusions in Six Pigs

<table>
<thead>
<tr>
<th>Control (before occlusion)</th>
<th>Occlusion I (mean ± SD)</th>
<th>Occlusion II (mean ± SD)</th>
<th>Occlusion III (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous</td>
<td>46.3 ± 13.7 ±</td>
<td>46.2 ± 14.7 ±</td>
<td>50.3 ± 15.7 ±</td>
</tr>
<tr>
<td>Arterial</td>
<td>36.0 ± 12.7 ±</td>
<td>37.1 ± 13.4 ±</td>
<td>41.9 ± 16.7 ±</td>
</tr>
<tr>
<td>Reperfusion (venous)</td>
<td>2 min</td>
<td>73.5 ± 19.0 ±</td>
<td>53.4 ± 10.9 ±</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>51.3 ± 17.1 ±</td>
<td>51.5 ± 11.3 ±</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>42.8 ± 10.8 ±</td>
<td>46.7 ± 12.8 ±</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>41.3 ± 12.0 ±</td>
<td>47.8 ± 15.0 ±</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>44.8 ± 14.2 ±</td>
<td>49.8 ± 15.8 ±</td>
</tr>
</tbody>
</table>

*p < 0.03. †p < 0.01. ‡p < 0.001.
Figure 6. Changes in base excess and pH in blood samples from great cardiac vein during reperfusion of three cycles of occlusion–reperfusion. The decrease in base excess and pH was most striking during the first reperfusion with respect to preocclusion levels (Preocl.).

Reperfusion, a brisk increase was observed, reaching the highest peak at 3 min. of 320 mol/liter kg, suggesting a massive K⁺ reentrance (36) because the Na⁺/K⁺ pump may remain preserved during ischemic periods of at least 15 min (37). More recently, Coronel et al. (33) found, also in the isolated perfused pig heart, similar extracellular K⁺ depletion during the first minute of reperfusion after a 10-min coronary occlusion. In our experiments, hypokalemia was lowest when the incidence of VF was highest, namely, at the first reperfusion and within the first 2 min. In view of the facilitating effect of hypokalemia in the development of VF (36,37,39,40), it is reasonable to suspect that this may be one potential explanation for the asymmetric distribution of VF in our experiments.

It is unclear whether the reelevation of ST segment consistently observed during the first minutes of the first reperfusion may in part relate to the regional hypokalemia. ST segment reelevation has also been recently documented during reperfusion after thrombolysis in some patients with acute myocardial infarction (41,42). Although its mechanism is not apparent, a possible explanation could be the striking action potential shortening documented by Coronel et al. (38) during reperfusion after a 10-min occlusion in the pig heart. The absence of this ST segment reelevation in subsequent reperfusions may be accounted for by the faster washout or lesser amount of metabolites released.

Implications. If we extrapolate these results to patients with unstable angina or acute myocardial infarction, where occlusion and reperfusion arrhythmias may supervene (43,44) and lead to sudden death (7,45,46), it is possible that repetitive episodes of ischemia could enhance rather than reduce the incidence of VF during a subsequent episode of ischemia. This could help to explain some instances of sudden death after intermittent episodes of ischemia (47,48) because it is possible that in a subsequent episode, the incidence of VF would not
only be higher but would also tend to occur earlier. Moreover, our findings may contribute to a better understanding of the enhanced risk for VF during myocardial infarction in patients with systemic hypokalemia because this may further lower regional extracellular K⁺ concentration during reperfusion.

References


