Exacerbation of Reperfusion Arrhythmias by Sudden Oxidant Stress

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A burst of free oxygen radical production has been demonstrated during the early moments of reperfusion, coincident with the onset of reperfusion arrhythmias, which can be attenuated by antioxidants. We have investigated whether a sudden burst of oxidant stress, superimposed on that occurring during reperfusion, can exacerbate reperfusion arrhythmias. Rat hearts (n = 12/group) were subjected to 12 minutes of aerobic perfusion; during the last 2 minutes, rose bengal (1 μmol/l) was added to the perfusion fluid. Then, regional ischemia was induced, and rose bengal-free perfusion was restored. After 5 minutes of ischemia, reperfusion was initiated for 5 minutes, and during the first 30 seconds of reperfusion, hearts were uniformly illuminated (8,500 lux) with green light (530–590 nm). The photoactivation of rose bengal trapped in the tissue, producing singlet oxygen and superoxide, resulted in an exacerbation of reperfusion arrhythmias. Thus, 92% of hearts developed ventricular premature beats, 83% ventricular tachycardia, and 33% ventricular fibrillation. In contrast, hearts with regional ischemia and reperfusion in the absence of rose bengal and/or illumination did not develop ventricular fibrillation; only one heart exhibited ventricular tachycardia, and the incidence of ventricular premature beats was lower (42–50%). Furthermore, the burst of oxidant stress shortened the time to onset of ventricular premature beats from 21.7±5.6 to 9.9±2.1 seconds. Additional studies revealed that rose bengal photoactivation without reperfusion was less arrhythmogenic compared with the combination of reperfusion plus photoactivation. These results demonstrate that a sudden burst of oxidant stress during the early moments of reperfusion can exacerbate the vulnerability to reperfusion arrhythmias. (Circulation Research 1990;67:481–489)

Using electron spin resonance techniques, it has been demonstrated that myocardial reperfusion results in a sudden burst of free oxygen radical production.1–3 A number of studies4–7 have suggested that the reactive oxygen intermediates, which are produced at this time, might contribute to the genesis of reperfusion arrhythmias. It has been proposed8 that the sudden burst of oxidant stress may injure membrane proteins and/or lipids and that this would lead to a perturbation of ionic balance and consequent electrical instability. In support of this hypothesis, we have recently reported9,10 that reactive oxygen intermediates (singlet oxygen and superoxide radicals) generated in isolated rat hearts by the photoactivation of rose bengal,11–14 can rapidly induce electrophysiological disturbances and arrhythmias. We have attributed these effects to oxidant stress from singlet oxygen since histidine, which quenches singlet oxygen, affords some protection against the electrophysiological changes and arrhythmias.9,15

Previously, we studied9,10,15,16 the effects of rose bengal photoactivation in aerobically perfused hearts. Although the arrhythmias and ultrastructural injury resembled those seen during severe ischemia and reperfusion, we did not use ischemia and reperfusion in the protocols. The objective of the present study was to exploit the capability of rose bengal photoactivation to produce a sudden burst of reactive oxygen intermediates in situ to assess whether additional oxidant stress, superimposed on the rat heart during the early moments of reperfusion, further increased its vulnerability to arrhythmias.

Materials and Methods

Animals and Reagents

Male Wistar rats (220–280 g body weight) were obtained from Bantin and Kingman, Humberstone, UK. Purified rose bengal (3',4',5',6'-tetrachloro-2,4,5,7-tetraiodofluorescein, MW 1,018) was obtained from Aldrich, Dorset, UK. The animal studies con-
formed to the guiding principles of the American Physiological Society.

**Perfused Heart Preparation**

Animals were anesthetized with diethyl ether and injected with sodium heparin (200 units i.v.). Thirty seconds later, hearts were excised and placed in cold (4°C) perfusion medium until contraction had ceased (approximately 15 seconds). Each heart was then cannulated via the aorta and perfused\(^\text{17}\) at a constant perfusion pressure of 100 cm H\(_2\)O.

**Perfusion Fluids**

Bicarbonate buffer (pH 7.4, 37°C), containing (in mmol/l) NaCl 118.5, NaHCO\(_3\) 25.0, KCl 3.2, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, CaCl\(_2\) 1.4, and glucose 11.1, was the standard perfusion fluid. This was gassed with 95% oxygen plus 5% carbon dioxide, and before use the perfusion fluid was filtered (pore size, 5 \(\mu\)m).

To prepare the rose bengal–containing perfusion fluid, 1 \(\mu\)mol rose bengal was added to each liter of perfusion fluid before filtration. The reservoir containing this solution, as well as all perfusion lines, were covered with aluminum foil to prevent interference by ambient light.

**Fiber Optic Illumination System**

As described previously,\(^\text{9,10}\) an illumination system was constructed from 200 fiber optic cables (each of 1-mm diameter) arranged in a uniform array around a specially constructed, temperature-regulated heart perfusion chamber. Each cable (100 cm long) delivered green light (530–590 nm) from two air-cooled 90-W dichroic light sources equipped with appropriate heat and interference filters. In the present study, the intensity of light delivered into the heart chamber was approximately 8,500 lux. In the absence of rose bengal, the illumination has no arrhythmogenic effects,\(^\text{9}\) and the myocardial temperature during illumination remains below 37.6°C.

**Induction of Ischemia and Reperfusion**

At the start of each experiment, a ligature was placed around the left anterior descending coronary artery at a point close to its origin. Both ends of the ligature were passed through a small plastic tube, which was then pressed against the artery. The resulting occlusion could be maintained for any desired period by clamping the plastic tube and the ligature. Reperfusion took place on removal of clamp and tube.

**Choice of Duration of Ischemia**

The vulnerability of the heart to reperfusion arrhythmias is critically dependent on the duration of the period of preceding regional ischemia.\(^\text{18}\) Since in the present study we were assessing whether a sudden burst of oxidant stress increased the vulnerability to reperfusion arrhythmias, it was necessary for the control group to show a low incidence of reperfusion arrhythmias. To achieve this, a 5-minute period of regional ischemia followed by a 5-minute period of reperfusion was selected.

**Experimental Time Course**

The protocols used for the present study are depicted in Figure 1. Each group consisted of 12 hearts, and the experiments were fully randomized. Group 1: Control. After 12 minutes of rose bengal–free perfusion, hearts were subjected to 5 minutes of regional ischemia followed by 5 minutes of reperfusion in the absence of rose bengal and illumination.

Group 2: Effects of rose bengal in the absence of illumination. After 10 minutes of rose bengal–free perfusion, the perfusion fluid was switched to one containing rose bengal (1 \(\mu\)mol/l), and perfusion was maintained for 2 minutes. At the end of this period, the left anterior descending coronary artery was ligated, and rose bengal–free perfusion was restored simultaneously. After 5 minutes of regional ischemia,
reperfusion, in the absence of illumination, was initiated and maintained for 5 minutes.

**Group 3:** In situ generation of reactive oxygen intermediates during the early moments of reperfusion. Hearts were subjected to the same experimental time course as in group 2 except that they were uniformly illuminated (8,500 lux) for the first 30 seconds starting from the moment of reperfusion.

**Group 4:** In situ generation of reactive oxygen intermediates in the absence of reperfusion. After 5 minutes of rose bengal-free perfusion, the perfusion fluid was switched to one containing rose bengal (1 µmol/l), and perfusion was maintained for 2 minutes. The left anterior descending coronary artery was then ligated, and rose bengal-free perfusion was restored simultaneously. After 5 minutes of regional ischemia, the hearts were uniformly illuminated (8,500 lux) for 30 seconds, and, instead of reperfusion, regional ischemia was maintained for another 5 minutes.

**Indexes Measured**

**Coronary flow and heart rate.** Coronary flow was measured by a timed collection of coronary effluent. Heart rate was calculated from the RR interval of the electrocardiogram.

**Identification and quantification of arrhythmias.** An electrocardiogram was recorded throughout the experiment via two silver electrodes attached to the ventricular apex and to the aortic cannula. This was analyzed for 1) the incidence, time to onset, and total number of ventricular premature beats (VPBs) (individual deflections in a run of ventricular tachycardia [VT] were not included as VPBs); and 2) the incidence, time to onset, and duration of VT and ventricular fibrillation (VF). Arrhythmias were defined and quantified in accordance with the Lambeth Conventions as follows. VPBs were defined as premature beats of ventricular origin, VT as four or more consecutive VPBs, and VF as a signal in which individual QRS deflections can no longer be distinguished from one another.

**Quantification of size of occluded zone.** The size of occluded zone was measured as described by Curtis and Hearse. At the end of each experiment, the heart with ligature released was perfused with disulfine blue (ICI, Cheshire, UK). The ligature was then retightened, and the heart was perfused with normal perfusion fluid. When the dye was washed out of the nonischemic zone (but remained in the occluded zone), perfusion was terminated and the heart was removed from the perfusion apparatus. The occluded and nonischemic zones were carefully dissected, lightly blotted, and weighed. The size of the occluded zone was expressed as a percentage of total ventricular weight.

**Exclusion Criteria**

Prospective exclusion criteria for this study demanded that hearts were excluded if 1) at the end of the first 10-minute period of perfusion, heart rate was lower than 280 beats/min or higher than 420 beats/min and/or coronary flow was less than 8.5 ml/min or more than 15 ml/min, 2) heart rate was less than 240 beats/min during the period of regional ischemia, 3) hearts exhibited any ventricular arrhythmias during the period before coronary artery ligation or in a 5-second period prior to reperfusion, or 4) the weight of the occluded zone was less than 40% or more than 50% of the total ventricular weight. A total of 54 hearts were entered in the study; two were excluded on functional grounds and four on the basis of size of the occluded zone.

**Statistics**

Statistical analysis was based on the guidelines described by Wallenstein et al. Gaussian-distributed variables were expressed as mean±SEM. A one-way analysis of variance was first carried out to test for any differences between the mean values of all groups. If a difference was established, the groups were compared by Tukey's test. An analogous procedure was followed for binomially distributed variables (e.g., incidence of VPBs). An overall $\chi^2$ test for a $2 \times n$ table was constructed, followed by a sequence of $2 \times 2 \chi^2$ tests using the Yates correction, to compare individual groups. A value of $p<0.05$ was considered significant.

**Results**

**Changes in Heart Rate and Coronary Flow**

Table 1 shows the changes in heart rate and coronary flow in each study group. Heart rate was unaffected by perfusion with rose bengal; however, coronary flow increased by 20–30%, and this persisted even after the return to rose bengal-free perfusion. Coronary artery ligation resulted in a reduction in heart rate (by 6–8%) and coronary flow (by 33–36%). After reperfusion, heart rate returned to its preischemic value, and there was a rapid reestablishment of coronary flow to its preischemic value or greater. Heart rate could be measured at 1 or 5 minutes after the onset of reperfusion and/or illumination, since some hearts did not develop arrhythmias or the duration of VT and VF in those with rhythm disturbances were relatively short. (These points will be discussed later.)

**Size of Occluded Zone**

In the present study, mean sizes of occluded zones in groups 1, 2, 3, and 4 were 44.7±0.5%, 44.6±0.7%, 43.3±0.7%, and 44.7±0.5% of the ventricular weight, respectively; there were no significant differences between these values.

**Arrhythmias**

**Group 1.** In this primary control group, hearts were subjected to regional ischemia (5 minutes) and reperfusion (5 minutes) in the absence of rose bengal and illumination. This allowed the assessment of the severity of arrhythmias during ischemia and reperfusion in the absence of additional oxidant stress. Table 2 shows that VT and VF did not occur in any heart
during ischemia or reperfusion and that a small number of VPBs occurred in 8% of hearts during ischemia and 42% of hearts during reperfusion. As shown in Figure 2, the VPBs that did occur during reperfusion had a mean time to onset of 32.0±5.9 seconds.

**Group 2.** This control group was included to assess whether rose bengal in the absence of illumination had any effects on vulnerability to arrhythmias. In this and subsequent groups, rose bengal was administered to the heart by perfusion for 2 minutes before coronary artery ligation. In this way, the compound was trapped in the ischemic zone during the period of regional ischemia but was washed out of the vascular space in the surrounding nonischemic tissue. As shown in Table 2 and Figure 2, there were few arrhythmias during ischemia and reperfusion; there were no statistically significant differences between groups 1 and 2.

**Group 3.** In this group, a burst of oxidant stress was imposed on the heart during the first 30 seconds of reperfusion. As can be seen from Table 2, and as would be expected, arrhythmias occurring in the 5-minute ischemic period before illumination were few, and their incidence was identical to that in groups 1 and 2. However, severe arrhythmias were observed during reperfusion, 92% of hearts exhibited VPBs, 83% VT, and 33% VF. Figure 3 shows a typical pattern of arrhythmias occurring during the early moments of reperfusion and illumination. This profile was similar to those observed during reperfusion after a short period of ischemia. Generally, VPBs developed first and deteriorated to VT or VF. In group 3, the total number of VPBs (Table 2) was up to 20 times greater than that in the two control groups (groups 1 and 2), and the mean time to onset of VPBs (Figure 2) was reduced to 9.9±2.1 seconds. When they occurred, both VT and VF were of short duration (the means of which were 2.2±0.5 and 6.0±2.1 seconds, respectively).

**Group 4.** In this group, hearts were perfused with rose bengal for 2 minutes, and after 5 minutes of regional ischemia they were illuminated for 30 seconds; however, in contrast to the preceding groups, reperfusion was not initiated. The objective of group 4 was to assess the extent to which arrhythmias would occur in

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**Table 1. Changes in Heart Rate and Coronary Flow**

<table>
<thead>
<tr>
<th>Group</th>
<th>RB</th>
<th>IL</th>
<th>RP</th>
<th>10 min</th>
<th>11 min</th>
<th>13 min</th>
<th>16 min</th>
<th>18 min</th>
<th>22 min</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>+</td>
<td>355±10</td>
<td>342±10</td>
<td>316±11</td>
<td>308±13</td>
<td>331±10</td>
<td>348±9</td>
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<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td>10.2±0.4</td>
<td>9.6±0.4</td>
<td>6.4±0.3</td>
<td>6.0±0.2</td>
<td>11.7±0.5</td>
<td>10.6±0.5</td>
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<tr>
<td>CF (ml/min)</td>
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<td></td>
<td></td>
<td>10.2±0.4</td>
<td>9.6±0.4</td>
<td>6.4±0.3</td>
<td>6.0±0.2</td>
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<td>10.6±0.5</td>
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<td>+</td>
<td>355±7</td>
<td>347±6</td>
<td>325±8</td>
<td>333±8</td>
<td>337±10</td>
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<td>HR (beats/min)</td>
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<td></td>
<td>10.0±0.2</td>
<td>12.6±0.5*</td>
<td>8.5±0.4*</td>
<td>8.2±0.4*</td>
<td>13.4±0.4†</td>
<td>13.1±0.4*</td>
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<tr>
<td>CF (ml/min)</td>
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<td>10.1±0.2</td>
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<td>8.4±0.3*</td>
<td>8.1±0.3*</td>
<td>13.7±0.4†</td>
<td>13.5±0.4*</td>
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<tr>
<td>CF (ml/min)</td>
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<td>10.1±0.2</td>
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<td>8.4±0.3*</td>
<td>8.1±0.3*</td>
<td>13.7±0.4†</td>
<td>13.5±0.4*</td>
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<td>353±12</td>
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<td>326±15</td>
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<td>HR (beats/min)</td>
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<td></td>
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<td>8.8±0.4*</td>
<td>8.5±0.4*</td>
<td>8.5±0.3*</td>
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<td>CF (ml/min)</td>
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<td>10.6±0.4</td>
<td>13.0±0.5*</td>
<td>8.8±0.4*</td>
<td>8.5±0.4*</td>
<td>8.5±0.3*</td>
<td>8.1±0.3*</td>
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</table>

Values are mean±SEM; n=12 hearts/group. RB, rose bengal (1 μmol/l) perfused for 2 minutes before coronary artery ligation; IL, illumination (8,500 lux) during the first 30 seconds of reperfusion; RP, reperfusion; HR, heart rate; CF, coronary flow.

*p<0.01 vs. group 1.

†p<0.05 vs. group 1.

**Table 2. Arrhythmias During Ischemia and Reperfusion in Groups 1, 2, and 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>RB</th>
<th>IL</th>
<th>RP</th>
<th>Incidence (VPBs)</th>
<th>Total VPBs</th>
<th>Incidence (VF)</th>
<th>Total VF</th>
<th>Incidence (VT)</th>
<th>Total VT</th>
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<td></td>
<td>+</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0.3±0.3</td>
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<td>+</td>
<td></td>
<td>+</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0.2±0.2</td>
<td>50</td>
<td>8</td>
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<td>3</td>
<td></td>
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<td>+</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0.3±0.2</td>
<td>92*</td>
<td>83‡‡</td>
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</table>

Values are mean±SEM; n=12 hearts/group. RB, rose bengal (1 μmol/l) perfused for 2 minutes before coronary artery ligation; IL, illumination (8,500 lux) during the first 30 seconds of reperfusion; RP, reperfusion; VPBs, ventricular premature beats; VT, ventricular tachycardia; VF, ventricular fibrillation.

*p<0.05 vs. group 1.

†p<0.01 vs. group 1.

‡p<0.01 vs. group 2.
FIGURE 2.  The effect of rose bengal (RB) photoactivation on the mean time to onset of ventricular premature beats (VPBs). Group 1: Control with no RB and no illumination (IL). Group 2: Control with RB (1 μmol/l) but no IL. Group 3: RB (1 μmol/l) photoactivation; IL, 8,500 lux. Each point represents the time that the first VPB was observed in each heart; the bars indicate the mean and SEM for each group (n=12 hearts/group). RP, reperfusion. *p<0.01 vs. group I; †p<0.05 vs. group 2.

hearts that had been illuminated in the absence of reperfusion and remained regionally ischemic.

The results show that the severity of arrhythmias was not as great as in group 3 (which was illuminated and reperfused) but was greater than in groups 1 and 2. Thus, in Figures 4A and 4B, the time to onset of VPBs and VT in group 3 (with reperfusion) is compared with that in group 4 (without reperfusion). Figure 5 illustrates the time profile for the incidence of ventricular arrhythmias (VPBs, VT, or VF quantified every 30 seconds from the onset of illumination). In group 3, in which the highest incidence of arrhythmias was observed during illumination, the incidence decreased rapidly after illumination was stopped. In contrast, in group 4, although there was a small increase during illumination, the incidence progressively increased during the ensuing 5 minutes of ischemia.

As shown in Table 3, most hearts in groups 3 and 4 developed VPBs and VT (92% and 83% versus 100% and 92%, respectively) during a 5-minute period from the onset of illumination. However, the mean time to onset of these arrhythmias was delayed in group 4 (Figures 4A and 4B); VPBs occurred after 89.3±22.3 versus 9.9±2.1 seconds in group 3 and VT after 136.9±25.3 versus 9.9±1.2 seconds in group 3. This difference is particularly marked when the incidences of arrhythmias during the 30-second period of illumination are compared (Table 3). In group 3 (with reperfusion), 92% of hearts exhibited VPBs, 83% VT, and 33% VF, whereas in group 4 (without reperfusion) the values were 33%, 17%, and 0%, respectively.

The results with groups 3 and 4, while indicating that the photoactivation of rose bengal can enhance the genesis of arrhythmias even in the absence of reperfusion, suggest that the photoactivation procedure by itself in the absence of reperfusion might not be able to induce such severe arrhythmias as those seen in group 3 very shortly after reperfusion. They also suggest that the rapid increase of arrhythmias observed in group 4 in the later period of ischemia might be due to the extension of the ischemic interval from 5 to 10 minutes.

Discussion

These present results demonstrate that a burst of oxidant stress, generated in situ in myocardial tissue during the early moments of reperfusion, can exacerbate the arrhythmogenic consequences of reperfusion. This provides further evidence for the concept that oxidant stress during the first few minutes of reperfusion could be included in the list of

FIGURE 3. Continuous electrocardiographic recording obtained from a heart in group 3 that was perfused with rose bengal (1 μmol/l) for 2 minutes before coronary artery ligation (5 minutes) and illuminated (8,500 lux) during the first 30 seconds of reperfusion. This recording shows ventricular arrhythmias developing approximately 9 seconds from the onset of reperfusion and illumination (indicated by the arrow), VT, ventricular tachycardia; VF, ventricular fibrillation; VPBs, ventricular premature beats.
triggers that contribute to the rapid genesis of reperfusion arrhythmias.

Oxidant Stress and Arrhythmias

The concept that reactive oxygen intermediates may cause cellular damage leading to reperfusion arrhythmias has its origins in the work of Manning et al.\(^2\) and Woodward and Zakaria.\(^5\) Specifically, these and other investigators\(^6,7,22\) have demonstrated, both in vivo and in vitro, that antioxidants, free radical scavengers, and agents that interfere with cellular free oxygen radical production can reduce the vulnerability of the heart to VF during reperfusion after a transient (5–20-minute) period of regional ischemia. In addition, it has been shown that free radical generating systems can enhance vulnerability to arrhythmias\(^9\) and can also induce electrophysiological changes likely to lead to arrhythmias.\(^21–23\) Lending further support to the association between free oxygen radical production and the genesis of arrhythmias are the results of a number of electron spin resonance studies,\(^1–3\) which show that in several species, both in vivo and in vitro, a burst of radical production does occur in the myocardium during the early moments of reperfusion. The association between this and the antiarrhythmic effects of antioxidants is of course indirect and may be circumstantial. Furthermore, investigators from this laboratory\(^18,26\) have always stressed that free oxygen radicals are likely to be but one of several triggers that contribute to the ionic disturbances that underlie the genesis of arrhythmias.

The duration of VT and VF in the hearts subjected to a combination of reperfusion and superimposed oxidant stress (group 3) was relatively short (mean durations were approximately 2 and 6 seconds, respectively). This finding, together with our earlier observation\(^5,10\) that VT induced by the rose bengal photoactivation showed short repetitive bursts, might suggest that oxidant stress plays a role in the genesis of the reperfusion arrhythmias, but not their maintenance.

Value and Limitations of the Rose Bengal Photoactivation Model

One of the difficulties with most free radical generating systems, such as FeCl\(_3\) plus adenosine diphosphate, xanthine plus xanthine oxidase, or hydrogen peroxide,\(^6,27,28\) is that they must be prepared in perfusion solutions and are therefore actively generating
Mechanisms of bengal photoactivation

Although heart.

quences16 during ischemia and electrophysiological37 propose that in the presence of the tissue, it binds to tissue (and can be subsequently removed from the vascular space as in the present study), and it can be instantaneously activated by illumination. In this way we have, for the first time, been able to mimic, in situ, a transient burst of oxidant stress, perhaps similar to that occurring in the heart during the early moments of reperfusion.

Despite the advantages of the rose bengal photoactivation, it should be stressed that it produces singlet oxygen and superoxide and, from the results of previous studies,9,15 we have concluded that it is the singlet oxygen that is most likely to be the injurious species. Other studies3,27-32 have focused on superoxide, hydroxy radicals, or hydrogen peroxide as mediators of injury. However, singlet oxygen is known to be associated with certain disease processes,33 and it has also been demonstrated34 that histidine (a quencher of singlet oxygen) reduced the peroxidation of unsaturated lipids and the intensity of the oxygen paradox during reoxygenation of the heart. Although it is possible that, during reoxygenation or reperfusion of tissue, singlet oxygen can be formed via the reactions of other reactive oxygen intermediates35 or breakdown of lipid hydroperoxides,36 there is as yet no conclusive evidence that it is produced during myocardial ischemia or reperfusion (the very short lifetime of singlet oxygen would make such proof very difficult). For this reason, and as stressed in our previous studies,9,10 we do not propose singlet oxygen–induced injury as a model of ischemia and reperfusion, despite the fact that the electrophysiological37 and ultrastructural consequences18 are in many ways similar.

Possible Mechanisms

In a previous study,16 we have established that rose bengal photoactivation can lead to a redistribution of cellular calcium and to ultrastructural changes (contraction) that resemble those associated with severe injury during ischemia and reperfusion. In a study37 with isolated papillary muscles, it has been demonstrated that photoactivation of rose bengal induces a transient inotropic response (which can be overcome by caffeine) followed by a negative inotropic effect and the development of contracture. Associated with this is the occurrence of aftercontractions and oscillatory afterpotentials. These findings are suggestive of a progressive disturbance of intracellular calcium involving the sarcoplasmic reticulum. Using isolated sarcoplasmic reticulum calcium release channels, Cumming et al38 have shown that rose bengal photoactivation can initially increase the probability of the "channel open state" but eventually leads to closure of the channel and to molecular changes in its protein structure. Recently, a study39 with isolated rabbit ventricular cells revealed that oxidant stress generated by the rose bengal photoactivation decreases resting potassium conductance (which will increase vulnerability to ventricular automaticity) and induces transient inward currents (which indicates cellular calcium overload) leading to repetitive action potentials.

All of these observations would be consistent with earlier speculation40 that oxidant stress during the early moments of reperfusion might change the redox state of controlling groups on various carrier proteins. Thus, Reeves et al41 have shown how reactive oxygen intermediates can activate sarcoplasmic calcium, and Salamama et al42 have shown how the redox state of thiol groups on the sarcoplasmic reticulum calcium release channel control the activity of that channel.

In the context of the above findings and cited studies, we would propose that during the early moments of reperfusion the burst of oxidant stress (which is inadequately handled by endogenous antioxidant defenses) may lead to changes in the activity (activation or inactivation) of a number of membrane carrier proteins. Thus, at the end of ischemia and during the early moments of reperfusion when the cell is depolarized and sodium loaded, sodium-calcium exchange might be activated by oxidant stress. This, together with other changes in sarcoplasmic reticulum calcium handling, would lead to ionic imbalance, oscillatory sarcoplasmic reticulum

### Table 3. Arrhythmias During 5 Minutes From the Onset of Illumination and During Illumination in Groups 3 and 4

<table>
<thead>
<tr>
<th>Group</th>
<th>RB</th>
<th>IL</th>
<th>RP</th>
<th>VPBs</th>
<th>VT</th>
<th>VF</th>
<th>Total VPBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>92</td>
<td>83</td>
<td>33</td>
<td>24.9±5.6</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>100</td>
<td>92</td>
<td>8</td>
<td>46.9±8.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=12 hearts/group. RB, rose bengal (1 μmol/l) perfused for 2 minutes before coronary artery ligation; IL, illumination (8,500 lux): In group 3 during the first 30 seconds of reperfusion, in group 4, 30 seconds after 5 minutes of ischemia had elapsed; RP, reperfusion; VPBs, ventricular premature beats; VT, ventricular tachycardia; VF, ventricular fibrillation.

*p<0.05 vs. group 4.

*p<0.01 vs. group 4.
calciump release, oscillatory changes in membrane potential, and arrhythmias.

Concluding

The present study has shown that oxidant stress, superimposed for a very short period at the onset of reperfusion, can increase the vulnerability of the heart to arrhythmias during the first few minutes of reperfusion. Our results (in group 4) indicate that these events can occur to a lesser extent in the absence of reperfusion; however, it is the combination of reperfusion plus oxidant stress that is the most potent arrhythrogenic stimulus.

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