Activation of Cardiac Vagal Afferents in Ischemia and Reperfusion
Prostaglandins Versus Oxygen-Derived Free Radicals

Elena E. Ustinova, Harold D. Schultz

Abstract Myocardial ischemia and reperfusion can evoke excitation of cardiac vagal nerve endings and activation of a cardiogenic depressor reflex (Bezold-Jarisch effect). We postulate that oxygen-derived free radicals, which are known to be produced during prolonged ischemia and reperfusion, contribute to this afferent excitation. We recorded activity from 47 chemosensitive vagal afferent fibers in 31 rats; the endings of these fibers were located in the left ventricle. Chemosensitive endings were identified with topical applications of capsaicin (10 \( \mu \)g) to the surface of the heart. Reactivity of the endings to oxygen-derived free radicals was assessed by topical application of \( \text{H}_2\text{O}_2 \) (3 to 9 \( \mu \)mol). Activity of the vagal fibers was recorded during 30 minutes of occlusion of the left anterior descending coronary artery (LAD) and 10 minutes of subsequent reperfusion. The activity of chemosensitive endings within the ischemic zone increased in the first 2 minutes of LAD occlusion from 2.2\( \pm \)0.4 to 4.3\( \pm \)0.9 impulses per second (107\( \pm \)30\% increase, \( P<.05 \)). This increased activity waned after 3 to 5 minutes of occlusion. Endings outside the ischemic zone did not increase, their activity at the beginning of ischemia. Reperfusion caused a rapid elevation of activity only in chemosensitive fibers whose endings were found to respond to topical \( \text{H}_2\text{O}_2 \). The reperfusion-sensitive endings were located both within and outside the ischemic zone of the left ventricle. Indomethacin (5 mg/kg IV, 20 minutes before occlusion) effectively prevented activation of chemosensitive afferent endings at the beginning of LAD occlusion regardless of their sensitivity to \( \text{H}_2\text{O}_2 \), but had no effect on the activation at reperfusion. The antioxidant deferoxamine (20 mg/kg IV, 20 minutes before occlusion) had no effect on the activation of the chemosensitive fibers at the onset of ischemia, although it completely prevented their activation at reperfusion. We propose that there are two different mechanisms that activate chemosensitive afferent vagal fibers in the rat heart during ischemia and reperfusion. The first causes excitation of these endings at the onset of ischemia and is mediated by prostaglandin synthesis within the ischemic zone. The second mechanism leads to a more widespread activation of chemosensitive afferents in the left ventricle by 30 minutes of ischemia and at the moment of reperfusion and is mediated by oxygen-derived free radical formation. (Circ Res. 1994;74:904-911.)

Key Words: • cardiac chemosensitive endings • ventricular C fibers • oxygen radicals • prostaglandins • antioxidants • myocardial ischemia • reperfusion

Studies during the last two decades have suggested that myocardial ischemia represents a potent stimulus capable of exciting cardiac sensory endings, which project both vagal and sympathetic afferent fibers to the central nervous system. As a consequence, multiple reflexes mediated by vagal and sympathetic afferent limbs can occur; these reflexes, in turn, modify both vagal and sympathetic outflow.1,2,3,4,5

Studies by Thames and Minis6 and Zucker et al7 have shown that occlusion of the circumflex coronary artery in dogs for 5 minutes results in a decrease in arterial pressure and renal sympathetic nerve activity6 and inhibition of the baroreflex.7 Blockade of prostaglandin synthesis with indomethacin reversed these effects. The authors suggested that during ischemia, prostaglandins serve as a major stimulus to ventricular chemosensitive endings with sympathoinhibitory vagal afferent fibers. In addition, clinical studies8,9 have shown that reperfusion of the ischemic myocardium by thrombolysis in patients with acute myocardial infarction is associated with bradycardia and hypotension. It was assumed that these cardiodepressor effects during reperfusion are also the result of stimulation of sympathoinhibitory reflexes mediated by these vagal afferents from the left ventricle.

One may be compelled to attribute these reflex cardioinhibitory and vasodepressor effects of myocardial ischemia and reperfusion to stimulation of chemosensitive vagal afferent nerve endings in the left ventricle, mainly because stimulation of these afferent endings by substances such as prostaglandins and bradykinin10,11 produces similar sympathoinhibitory reflex responses.9 Nevertheless, neurographic evidence that these afferent endings are stimulated during myocardial ischemia and during reperfusion is scant. Recently, Coleridge et al10 found that two thirds of the chemosensitive vagal C-fiber endings in the left heart of dogs are stimulated within 10 to 30 seconds of coronary occlusion and that activation of these cardiac endings is unrelated to changes in cardiac pressures. To date, however, there have been no other neurographic studies to determine whether ventricular chemosensitive vagal endings respond to prolonged ischemia and reperfusion.

Although the studies described above suggest a reflexogenic role of prostaglandin production in the ischemic myocardium, another important metabolic event that occurs in ischemia, and especially in reperfusion, is oxygen-derived free radical activation.11 A major consequence of oxygen-derived free radical metabolism is peroxidation of lipid membranes. Several studies have suggested that free radical lipid peroxidation disrupts

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the selective permeability of cell membranes and activity of membrane-bound enzymes, particularly Na⁺,K⁺-ATPase and Ca²⁺-ATPase, leading to impaired contractile function of the heart and cardiac arrhythmias. Antioxidants have been shown to decrease lipid peroxidation in the myocardium after both short-term and prolonged ischemia, to significantly enhance the recovery of contractile function of the heart during the period after ischemia, and to reduce arrhythmias and the area of ischemic necrosis after infarction. Although progress is being made in our understanding of how free radicals affect the function and excitability of cardiac cells, little is presently known about their effect on the function of cardiac sensory nerves. In the accompanying article in this journal, we have shown that H₂O₂ and xanthine/xanthine oxidase can activate chemosensitive cardiac vagal afferents in rats. This effect was abolished by the antioxidants deferoxamine and dimethylthiourea (DMTU), which limit the formation of the hydroxyl radical. Furthermore, the ability of oxygen-derived free radicals to stimulate the chemosensitive endings was not mediated by the cyclooxygenase system. On the basis of this evidence, we hypothesize that production of reactive oxygen species during myocardial ischemia and reperfusion stimulates sensory nerve endings in the heart.

The aim of the present study was to examine the effect of acute myocardial ischemia and reperfusion on the activity of cardiac chemosensitive vagal afferents in rats and to determine whether prostaglandin and/or free radical production contributes to the activation of these afferents in response to ischemia and reperfusion. To achieve the latter goal, we examined the effect of an antioxidant, deferoxamine, and an inhibitor of prostaglandin synthesis, indomethacin, on the activity of cardiac vagal afferents in response to ischemia and reperfusion.

Materials and Methods

Young adult Sprague-Dawley rats (250 to 320 g, either sex) were anesthetized intraperitoneally with a mixture of 2% α-chloralose and 25% urethane in saline (5 mL/kg). The trachea was cannulated low in the neck, and the lungs were ventilated by a Harvard rat respirator (60 breaths per minute) with air supplemented with O₂. Body temperature was maintained at 37°C by a heating pad. Polyethylene catheters were inserted in a carotid artery and jugular vein for measurement of arterial pressure and administration of drugs, respectively. The chest was opened via a sternotomy, and a 2F microcatheter pressure transducer (Millar) was inserted into the left ventricle via a needle puncture through the apex for measurement of left ventricular pressure. A silk suture (5–0) was looped around the left anterior descending coronary artery (LAD) at its origin to produce occlusion (see “Protocols” below). Heart rate was measured by a cardiostethoscope triggered by the arterial pressure pulse. Arterial and tracheal pressures were measured by strain gauges. Heart rate, arterial, ventricular, and tracheal pressures, and nerve activities (see below) were recorded by a thermal recorder (Astro-Med MT 95000). Estimated fluid loss was replaced with intravenous administration of physiological saline at a rate of 4 to 6 mL/kg per hour.

Recording of Afferent Vagal Impulses

Fine slips of the left cervical vagus were covered with mineral oil and placed on a silver electrode. Impulses were amplified (Grass P511 amplifier), displayed on an oscilloscope (Gould 450), and fed into a rate meter (Frederick Haer Co) whose window discriminators were set to accept potentials of a particular amplitude. Impulses were counted by rate meter in 1-second bins. Bundles that had one, or at most two, easily distinguishable active fibers were used.

Identification of Cardiac Fibers

In the accompanying article involving the study of cardiac vagal afferent endings, we found that only the chemosensitive afferents could be activated with free oxygen radicals. Therefore, in the present study, we examined only chemosensitive fibers. We selected only those fibers whose endings could be located precisely in the left ventricle by a fine-tipped probe.

Conduction velocity was determined by measuring the latency between electrical stimulation of the receptive field and recording of the evoked potential and measuring the conduction distance between the receptive field and recording electrode. All afferent fibers used in the present study had conduction velocities of <2.5 m/s and thus were C fibers. The chemosensitive afferent fibers were identified by systemic and topical administration of capsaicin to the heart. Capsaicin was chosen as the test chemical because it is known to directly stimulate only chemosensitive C-fiber endings and not cardiovascular mechanoreceptors. This test chemical was injected intravenously in doses of 0.5 to 1.0 μg, and then if the fiber was activated, capsaicin was applied to the surface of the heart to locate the receptive field (see below). H₂O₂ (3 to 9 μmol) was also applied to the end to assess whether the chemosensitive fiber was also responsive to the generation of oxygen-derived free radicals. Typically, the chemosensitive fibers exhibited an irregular discharge pattern unrelated to the cardiac cycle and were relatively unresponsive to changes in cardiac pressures.

At the end of the experiment, Monastral blue dye was injected intravenously during LAD occlusion to determine the size and location of the ischemic area. Afferent fibers were grouped with respect to the location of the ending in relation to the ischemic zone and with respect to the sensitivity of the ending to topical H₂O₂ (3 to 9 μmol). All recorded fibers from these experiments were thus subdivided into four groups: (1) H₂O₂-sensitive fibers with the ending within the ischemic zone (n=5), (2) H₂O₂-insensitive fibers with the ending within the ischemic zone (n=4), (3) H₂O₂-sensitive fibers with the ending in the nonischemic area of the ventricle (n=6), and (4) H₂O₂-insensitive fibers with the ending in the nonischemic area of the ventricle (n=4).

Drug Administration

A small circle of filter paper 3 mm in diameter was placed on the surface of the heart above the receptive field, and 10 μL of capsaicin (0.1 to 1 mg/mL in saline vehicle; total dose, 1 to 10 μg) was applied for 10 seconds. H₂O₂ was applied to the filter paper in concentrations of 10% to 30% in distilled H₂O₂ 10 μL of this solution was equivalent to doses of 3 to 9 μmol, respectively. These doses were shown to effectively activate chemosensitive vagal endings in the heart in a previous study. After each application, the paper was removed, and the surface of the heart was washed with warm saline. All drugs were obtained from Sigma Chemical Co.

Protocols

The specific questions that we addressed in the present study were as follows: (1) Do ischemia and reperfusion have similar effects on the activity of ventricular chemosensitive afferents? To address this question, we studied the effect of myocardial ischemia produced by occlusion of the LAD for 30 minutes and a subsequent 10-minute reperfusion (referred to as “Protocols” below) on the activity of the cardiac fibers. Afferent fibers were subdivided into four groups, as explained above. (2) Are the effects of ischemia and reperfusion on the activity of cardiac afferent endings decreased or abolished by cyclooxygenase blockade? In a separate group of rats, the afferent...
TABLE 1. Activation of Chemosensitive Cardiac Vagal C Fibers in Ischemia and Reperfusion

<table>
<thead>
<tr>
<th>Sensory ending within the ischemic zone</th>
<th>Total Fibers</th>
<th>Early*</th>
<th>Late†</th>
<th>Fibers Activated During Rp</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O₂ sensitive (group 1)</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>H₂O₂ insensitive (group 2)</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sensory ending outside the ischemic zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O₂ sensitive (group 3)</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>H₂O₂ insensitive (group 4)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>9</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

LCO indicates left anterior coronary occlusion; Rp, reperfusion.
The criterion for activation of a fiber was an increase in maximal activity by 50% above baseline.
*First 2 minutes of occlusion.
†Last minute of 30-minute period of occlusion.

Response to Ischemia and Reperfusion

Table 1 illustrates the number of fibers obtained from the first series of experiments, in which afferent responses to ischemia and reperfusion were examined without other experimental manipulations. Among 19 fibers tested with 30 minutes of LAD occlusion and 10 minutes of reperfusion, each of 9 fibers with their endings within the ischemic zone was activated during the first 1 to 2 minutes of the ischemic period. These 9 fibers comprised both H₂O₂-sensitive and H₂O₂-insensitive endings. Ten endings that were not in the ischemic zone, both H₂O₂ sensitive and H₂O₂ insensitive, were not activated at the beginning of coronary occlusion. Thus, all chemosensitive fibers tested that had endings within the ischemic zone were activated at the onset of ischemia. On the other hand, only the fibers that were H₂O₂ sensitive were activated at the beginning of reperfusion. The response to reperfusion was not correlated with the location of the endings with respect to the ischemic zone (Table 1, Fig 1).

Fig 1A illustrates that the activity of the fibers from group 1 (H₂O₂-sensitive fibers with endings within the ischemic zone) increased during the first 2 minutes of LAD occlusion from 2.2±0.4 to 4.3±0.9 imp/s (107±30% increase, P<.05). The elevation in activity subsided after 3 to 6 minutes of occlusion but remained above the control level at the end of the ischemic period. Reperfusion caused a second rapid elevation of activity from 3.5±0.7 imp/s at the end of the ischemic period to 5.9±0.9 imp/s (183±32% increase from control, P<.01) at the second minute of reperfusion. Activation during reperfusion lasted for 1 to 5 minutes and after 5 minutes gradually returned to the basal level. Fig 2 illustrates the activity of an individual fiber from this group during ischemia and reperfusion.

Analysis of Data

Reported values are mean±SEM. The firing rate of vagal fibers was calculated as the average number of impulses per second (imp/s) over a period of 20 seconds. Fiber activity was determined over 20 seconds of maximal activity at the final minute of the control period, during the first 2 minutes and the last minute of coronary occlusion, and during the first 2 minutes and 10th minute of reperfusion. Because of the individual variability in the control activity of different fibers, the neural responses were also expressed as percent change from the baseline. Differences among groups were determined by ANOVA for repeated measures, and differences between means were isolated by the Bonferroni correction for multiple t tests. Student’s paired and unpaired t tests were used for single comparisons. Statistical significance was accepted at P<.05.

Results

All vagal fibers that were chosen for study in these experiments were capsaicin sensitive, ie, chemosensitive fibers, and were located in the left ventricle. Activity from 47 chemosensitive fibers was recorded in 31 rats. Fifteen of the recordings were obtained from filaments with only one active fiber. In the other 16 recordings, two active fibers were present, but differences in spike heights permitted activity in the individual fibers to be determined separately. Topical application of capsaicin (10 μg) to the receptive field increased their activity from 2.4±0.2 to 8.0±0.6 imp/s (310±36% increase, P<.001). Thirty of these 47 endings (64%) also responded to the topical application of H₂O₂. The average activity of these fibers increased from 2.3±0.2 to 5.8±0.6 (196±29% increase, P<.01). All of the fibers exhibited an irregular discharge pattern unrelated to the cardiac cycle and were insensitive to changes in cardiac pressures.
Fibers from group 2 (H$_2$O$_2$-insensitive fibers with endings within the ischemic zone) increased their activity during the first 2 minutes of LAD occlusion from 3.4±0.4 to 6.8±0.8 imp/s (104±18% increase, P<.01). Activity gradually returned to the initial level by the end of the ischemic period. Reperfusion did not cause a change in fiber activity (Fig 1B). Fig 3 illustrates the activity of an individual fiber from this group during ischemia and reperfusion.

Fibers from group 3 (H$_2$O$_2$-sensitive fibers with endings outside the ischemic zone) were not activated at the beginning of the ischemic period. Their activity started to increase gradually after 10 to 12 minutes of LAD occlusion and reached a significant value 2 minutes after occlusion of the LAD; and C, 1 minute after reperfusion. Fig 2 shows the final minute of the control period; B, 2 minutes after occlusion of the LAD; and C, 1 minute after reperfusion. IF indicates impulse frequency (impulses per second [imp/s]); AP, action potentials; and LVP, left ventricular pressure.
occlusion from 2.5±0.9 imp/s at the end of the control period to 4.4±0.7 imp/s (97±32% increase, P<.05) at the end of 30 minutes of occlusion. Reperfusion caused an additional elevation of activity to 5.5±0.9 imp/s, which lasted for 1 to 5 minutes (Fig 1C).

Fibers from group 4 (H₂O₂-insensitive fibers with endings outside the ischemic zone) did not alter their activity during ischemia and reperfusion (Fig 1D).

**Effect of Indomethacin**

To test the hypothesis that prostaglandins are responsible for activation of the chemosensitive fibers during ischemia and reperfusion, we examined the responses of afferents from groups 1 through 3 to 30 minutes of LAD occlusion and 10 minutes of reperfusion in the presence of indomethacin (5 mg/kg IV). Afferent responses of the indomethacin-treated groups to ischemia and reperfusion were compared with those of the untreated groups described above (Fig 4).

Indomethacin abolished afferent responses at the onset of ischemia but had no effect on the pattern of activation during reperfusion (Fig 4). Thus, in indomethacin-treated rats, the activity of chemosensitive fibers with endings within the ischemic zone (groups 1 and 2) during the first 2 minutes of LAD occlusion did not differ from that during the last minute of their control period (Fig 4A and 4B). The level of activity in groups 1 and 2 during the first 2 minutes of LAD occlusion was significantly less in indomethacin-treated than in untreated rats (13±8% versus 107±18% increase, P<.05). However, by the end of the 30-minute ischemic period, the activity of H₂O₂-sensitive fibers (groups 1 and 3) in indomethacin-treated rats increased above the control level (65±22% and 80±19%, respectively; P<.05) and continued to increase further during reperfusion (Fig 4A and 4C). The afferent responses during prolonged ischemia and during reperfusion in indomethacin-treated rats did not differ from those observed in untreated animals.

In the present experiments, indomethacin had no effect on left ventricular systolic pressure and on the incidence and duration of arrhythmias in response to ischemia and reperfusion (Table 2).

**Effects of Antioxidants**

Since activation of chemosensitive endings in reperfusion correlated with their ability to respond to the application of H₂O₂ (groups 1 and 3), we studied the effect of the antioxidant deferoxamine (20 mg/kg IV) on the activity of chemosensitive fibers in ischemia and reperfusion (Fig 5).

Fig 5 illustrates that deferoxamine, unlike indomethacin, did not affect afferent responses at the onset of ischemia, but it completely prevented activation during

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**TABLE 2. Effect of Deferoxamine and Indomethacin on Left Ventricular Systolic Pressure and Arrhythmias in Ischemia and Reperfusion**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>LVSP, mm Hg</th>
<th>Premature Beats</th>
<th>Ventricular Fibrillation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-Minute Rp</td>
<td>Animals, %</td>
<td>No. of Beats</td>
</tr>
<tr>
<td>Untreated (n=10)</td>
<td>Control</td>
<td>80±8.6</td>
<td>86±4</td>
</tr>
<tr>
<td></td>
<td>5-Minute LCO</td>
<td>80±6.4</td>
<td>86±4</td>
</tr>
<tr>
<td>Indomethacin (n=5)</td>
<td>Control</td>
<td>74±3.3</td>
<td>79±4</td>
</tr>
<tr>
<td></td>
<td>5-Minute LCO</td>
<td>74±3.3</td>
<td>79±4</td>
</tr>
<tr>
<td>Deferoxamine (n=5)</td>
<td>Control</td>
<td>90±8.6</td>
<td>98±6</td>
</tr>
</tbody>
</table>

LVSP indicates left ventricular systolic pressure; Rp, reperfusion; and LCO, left anterior coronary occlusion.

*P<.05 and †P<.001 compared with control value.
reperfusion. Thus, in deferoxamine-treated rats, chemosensitive fibers with endings within the ischemic zone (groups 1 and 2) increased their activity during the first 2 minutes of LAD occlusion. This level of activity during the first 2 minutes of LAD occlusion in deferoxamine-treated rats did not differ from that observed in untreated rats (Fig 5A and 5B). However, H$_2$O$_2$-sensitive fibers (groups 1 and 3) in deferoxamine-treated rats failed to increase their activity above the control level in response to 30 minutes of coronary occlusion and to reperfusion (Fig 5A and 5C). The level of activity in groups 1 and 3 during the first 2 minutes of reperfusion was significantly less in deferoxamine-treated than in untreated rats (37±17% versus 183±32%, P<.01). The difference between deferoxamine-treated and untreated rats at 30 minutes of ischemia was not significant for group 1 fibers.

Administration of deferoxamine had no effect on left ventricular systolic pressure during ischemia and reperfusion. At the same time, this antioxidant significantly decreased the number of premature beats as well as the incidence and duration of ventricular fibrillation at the onset of reperfusion (Table 2). This observation is in accordance with the data of other studies that have shown the protective effect of deferoxamine in prolonged ischemia and reperfusion.16,17

In three additional H$_2$O$_2$-sensitive fibers with endings located within the ischemic zone (group 1), we studied the effect of DMTU (10 mg/kg IV) on their activity during ischemia and reperfusion. DMTU completely abolished activation of the fibers at the end of the ischemic period and at reperfusion. The average maximal activity of these fibers was 1.3±0.3 imp/s before LAD occlusion, 3.5±0.2 imp/s at the second minute of LAD occlusion, 1.6±0.3 imp/s at the end of the 30-minute ischemic period, and 1.1±0.5 imp/s after the first minute of reperfusion.

Discussion
The results of the present study demonstrate that regional ischemia produced by occlusion of the LAD in rats causes rapid activation of chemosensitive vagal endings located within the ischemic area. The same correlation was found by Coleridge et al.,10 who recorded impulses from afferent vagal endings in the left ventricle in dogs after the occlusion of the LAD or left circumflex artery. They found that two thirds of the chemosensitive C-fiber endings in the left heart were stimulated within 10 to 30 seconds of coronary occlusion. Most of the responsive chemosensitive endings were stimulated by occlusion of one coronary artery but not by occlusion of the other, depending on their location in the vascular territory supplied by the artery.

We examined whether afferent responses to coronary occlusion were mediated by prostaglandins, since the ischemic myocardium releases prostaglandins22 that are known to stimulate these nerve endings in dogs7 and to evoke a cardiogenic depressor reflex mediated by these afferents.9 We found, in rats, that inhibition of prostaglandin synthesis with indomethacin abolished activation of chemosensitive endings within the ischemic zone at the onset of LAD occlusion.

Thames and Minis15 found that occlusion of the circumflex coronary artery in dogs for 5 minutes resulted in a decrease in arterial pressure and renal sympathetic nerve activity. After treatment with indomethacin or meclofenamate, coronary occlusion resulted in significantly less inhibition of renal nerve activity. Similarly, Zucker et al.8 showed that the inhibition of the baroreflex during acute (5- to 10-minute) occlusion of the circumflex coronary artery in dogs could be abolished by either vagotomy or indomethacin. These studies have provided indirect evidence that prostaglandins released during myocardial ischemia stimulate cardiac vagal afferent endings, which, in turn, inhibit sympathetic outflow and attenuate the baroreflex. This assumption is supported by our experiments, based on the direct recordings from cardiac chemosensitive vagal afferents. However, our results also indicate that prostaglandins are important for activation of these afferents only at the onset of ischemia. Thus, our results suggest that prostaglandins play a functional role in the activation of cardiogenic reflexes during only brief periods of ischemia, as observed in these previous studies.
Sustained recordings of single afferent fibers during 30 minutes of coronary occlusion and 10 minutes of reperfusion allowed us to observe a second wave of activation that developed at the end of the ischemic period and, even more prominently, at the beginning of reperfusion. This activation was not abolished with indomethacin and thus was mediated by factors other than cyclooxygenase products. Only chemosensitive fibers that were found to be responsive to chemically induced formation of free radicals (by application of H$_2$O$_2$ to the heart) increased their activity at the end of the ischemic period and during reperfusion. Thus, it is quite likely that this activation was mediated by oxygen-derived free radicals.

We found that activation of cardiac vagal afferent endings in late ischemia and at reperfusion could be abolished by the antioxidants deferoxamine and DMTU. Deferoxamine is known to prevent the formation of hydroxyl radicals,10,11 and DMTU is a specific scavenger of hydroxyl radicals.12,13 Stahl et al14 reported that these antioxidants prevented reflex activation of the cardiovascular system in cats, which was mediated by abdominal sympathetic afferents in response to H$_2$O$_2$. These investigators subsequently demonstrated, using neurographic recordings, that abdominal visceral sympathetic afferents can be activated by H$_2$O$_2$ and by abdominal ischemia and that DMTU can decrease this activation.20 In our accompanying article,20 we found that, in rats, both deferoxamine and DMTU abolished activation of cardiac chemosensitive vagal afferents in response to application of xanthine/xanthine oxidase and H$_2$O$_2$ to the heart. Thus, there is supportive evidence that deferoxamine prevented activation of chemosensitive afferent endings in the heart during prolonged ischemia and at reperfusion by limitation of oxygen-derived free radical formation.

Antioxidants had no effect on the activation of the endings at the beginning of coronary occlusion, which suggests that oxygen-derived free radicals do not contribute to the excitation of cardiac chemosensitive endings in early ischemia. Several studies have shown that enhanced formation of free radicals does not begin immediately after the onset of ischemia. In the rat heart, an increase in hydroxyl radical production begins ~10 minutes after occlusion of the LAD,26,27 and the activity of antioxidant enzymes decreases 20 to 30 minutes thereafter.28 Nevertheless, 10 to 15 minutes of coronary occlusion in rats is sufficient to lead to a burst of free radical production during subsequent reperfusion.29,30

An unexpected finding of the present study was that all of the H$_2$O$_2$-sensitive endings found in the left ventricle were stimulated by prolonged ischemia and by reperfusion regardless of their location in relation to the ischemic zone. Because these afferent responses were abolished by deferoxamine, these results would suggest that oxygen-derived free radical formation is enhanced throughout the left ventricle in response to coronary occlusion and reperfusion. In support of this notion, other studies have shown that occlusion of the LAD in the rat decreases the activity of antioxidant enzymes and increases free radical formation in both ischemic and nonischemic areas of the heart.28,31 Similarly, depressed contractility of the isolated right atrium was observed after occlusion of the left coronary artery.32 This effect could be prevented by pretreatment with the antioxidant dibonol.

The mechanisms responsible for enhancement of free radical formation in the nonischemic myocardium during acute ischemia are not yet well understood. In addition to partial inactivation of endogenous enzymatic antioxidant systems, myocardial ischemia produces an excessive release of catecholamines,33 which are known to increase energy demand of the tissue and to activate lipases and phospholipases, i.e., metabolic changes that facilitate free radical peroxidation in the myocardium.34 Auto-oxidation of norepinephrine also results in free radical formation.35 In the present study, one could speculate that these metabolic changes enhanced free radical production in the nonischemic areas of the myocardium during the 30-minute period of ischemia. By contrast, in the ischemic zone, the lack of oxygen may limit activation of free radical production during ischemia, and a more marked increase in free radical production would occur at the beginning of reperfusion. This hypothesis could explain why the activity of H$_2$O$_2$-sensitive endings in the nonischemic zone of the left ventricle gradually increased over the course of 30 minutes of ischemia, whereas activation of H$_2$O$_2$-sensitive endings within the ischemic zone occurred more prominently at the onset of reperfusion.

The coronary chemoreflex, a cardioinhibitory and vasodepressor reflex characterized by bradycardia and hypotension2 and by evoked cholinergic coronary vasodilatation,36 is evoked by stimulation of chemosensitive vagal endings in the left ventricle. During thrombolytic therapy in humans, similar types of cardiovascular events commonly occur at the onset of myocardial ischemia37,38 and also during reperfusion of acute myocardial infarcts.5,6 The results of the present study, using electroneurographic recordings in rats, corroborate the notion that these reflex changes are initiated at the onset of myocardial ischemia by the stimulation of chemosensitive vagal afferents in response to production and release of prostaglandins from the ischemic tissue.3,4 Our results further suggest that during more prolonged periods of ischemia and during reperfusion, these reflex responses are more likely to be mediated by these afferents in response to oxygen-derived free radical production.

The functional significance of the effects of myocardial ischemia and reperfusion on cardiac chemosensitive vagal afferents remains unresolved. Needleman22 speculated that the increased parasympathetic tone and decreased sympathetic tone reflexly evoked by these afferents in response to prostaglandins are beneficial to the ischemic myocardium. Thus, the bradycardia, decreased contractility, and peripheral vasodilatation would decrease the work of the heart and reduce oxygen demand of the compromised myocardium. Furthermore, the cholinergic-mediated increase in coronary blood flow would increase oxygen delivery. The functional implications of these reflex changes at the onset of reperfusion of the ischemic myocardium are less clear, but certainly the increased parasympathetic drive could help to stabilize the excitability of the myocardium and impede potentially dangerous arrhythmias that are known to occur with myocardial reperfusion.11,15 Thus, despite the pronounced detrimental effects of free radicals on the function and excitability of cardiac myocytes,15-17 the stimulatory effect of these reactive oxygen species on chemosensitive vagal reflexes during reperfusion could be of some benefit.
In summary, our data indicate that there are two different mechanisms that activate cardiac chemosensitive vagal afferent fibers during myocardial ischemia and reperfusion in rats. The first causes activation of these endings within the ischemic zone at the onset of ischemia and is mediated by prostaglandin synthesis. The second mechanism leads to more widespread activation of cardiac chemosensitive afferents by 30 minutes of ischemia and at the moment of reperfusion and is mediated by free radical formation. Further studies will be required to determine the potential importance of these two mechanisms in mediating the reflex bradycardia, vasodilatation, and sympathoinhibition that accompany myocardial ischemia and reperfusion in humans.

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