Hypoxic reperfusion of the ischemic heart and oxygen radical generation

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Hypoxic reperfusion of the ischemic heart and oxygen radical generation. Am J Physiol Heart Circ Physiol 290: H341–H347, 2006. First published August 26, 2005; doi:10.1152/ajpheart.00223.2005.—Postischemic myocardial contractile dysfunction is in part mediated by the burst of reactive oxygen species (ROS), which occurs with the reintroduction of oxygen. We hypothesized that tissue oxygen tension modulates this ROS burst at reperfusion. After 20 min of global ischemia, isolated rat hearts were reperfused with temperature-controlled (37.4°C) Krebs-Henseleit buffer saturated with one of three different O2 concentrations (95, 20, or 2%) for the first 5 min of reperfusion and then changed to 95% O2. Additional hearts were loaded with 1) allopurinol (1 mM), a xanthine oxidase inhibitor, 2) diphenyleneiodonium (DPI; 1 µM), an NAD(P)H oxidase inhibitor, or 3) Tiron (10 mM), a superoxide scavenger, and were then reperfused either with 95% or 2% O2 for the first 5 min. ROS production and tissue oxygen tension were quantitated using electron paramagnetic resonance spectroscopy. Tissue oxygen tension was significantly higher in the 95% O2 group, however, the largest radical burst occurred in the 2% O2 reperfusion group (P < 0.001). Recovery of left ventricular (LV) contractile function and aconitase activity during reperfusion were inversely related to the burst of radical production and were significantly higher in hearts initially reperfused with 95% O2 (P < 0.001). Allopurinol, DPI, and Tiron reduced the burst of radical formation in the 2% O2 reperfusion groups (P < 0.05). Hypoxic reperfusion generates an increased ROS burst originating from multiple pathways. Recovery of LV function during reperfusion is inversely related to this oxygen radical burst, highlighting the importance of myocardial oxygen tension during initial reperfusion.

reactive oxygen species; contractile function; cardiac arrest

REPERFUSION, FOLLOWING GLOBAL ISCHEMIA of the heart, restores oxygen delivery to the ischemic tissue. During ischemia, there is a strong reductive pressure, which, with the reintroduction of oxygen, results in the burst of reactive oxygen species (ROS), which are thought to primarily be responsible for the postischemic myocardial contractile dysfunction (2, 4, 5). This phenomenon of postischemic contractile dysfunction may result from either cell injury or myocardial stunning (6), or a combination of both. This postischemic myocardial dysfunction is seen after a number of clinical syndromes including both regional (acute myocardial infarction) and global (cardiac arrest) myocardial ischemia (15, 20).

ROS, particularly superoxide, may be generated from multiple sources and mechanisms depending on the duration of preceding ischemia. Earlier studies identified endothelial cells as a major source of ROS at reperfusion that could be inhibited by xanthine oxidase blockers (31) and react with iron to form the very reactive hydroxyl radical (30). After prolonged periods of ischemia sufficient to result in necrosis, a major source of ROS appears to be neutrophils, which can be blocked with anti-neutrophil interventions causing a reduction of infarct size (7). However, after shorter periods of ischemia resulting in myocardial stunning but not necrosis, the major source of the ROS burst at reperfusion appears to be non-neutrophil-mediated (3). Other sources of the ROS burst at reperfusion include NAD(P)H oxidases, mitochondria (particularly complexes I and III), cyclooxygenase/lipoxygenase, and cytochrome P-450. Within the rat heart, xanthine oxidase is a significant source of oxygen free radicals upon reperfusion (26).

Controlled reperfusion of the ischemic myocardium has been advocated under conditions of surgically induced ischemia with cardioplegia, including controlled reoxygenation (13, 21). In the setting of cardiac arrest, ventilation with high (FiO2 1.0) or normal oxygen levels (FiO2 0.21) during resuscitation in a rat model failed to show any difference in neurological outcome (18). Similarly, hypoxic ventilation during cardiac arrest resuscitation in a swine model failed to show any neurological outcome difference compared with normoxic ventilation (33). However, in a canine model, hyperoxic (FiO2 1.0) ventilation during cardiac arrest resuscitation was associated with poorer neurological outcome compared with animals ventilated with room air (FiO2 0.21) (34). Likewise, in a rabbit model of normothermic renal ischemia, hyperoxic reperfusion increased renal dysfunction (32). From the latter two studies there is some evidence that hyperoxic reperfusion may exacerbate posts ischemic organ dysfunction. However, this has not been systematically examined in the reperfused ischemic heart.

We hypothesize that tissue oxygen tension during the initial reperfusion of the ischemic myocardium modulates early ROS generation. In this study we used the globally ischemic Langendorff rat heart and controlled oxygen concentration in the perfusate during reperfusion to determine ROS formation at reperfusion, the likely sources of ROS production, and the correlation with postischemic myocardial contractile and bioenergetic functions. The perfused isolated heart was used to tightly control reperfusion conditions and allow tissue measurement of oxygen tension and ROS with the use of electron paramagnetic resonance (EPR) spectroscopy. We observed a graded response of ROS production inversely related to the tissue oxygen tension at reperfusion, with the largest ROS burst associated with the most hypoxic tissue oxygen tension.

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METHODS

Langendorff-perfused heart preparation. Male Sprague-Dawley rats (350–450 g) supplied by Harlan (Indianapolis, IN) were used in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the approval of the Ohio State University Laboratory Animal Resources Committee. Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). The right superficial jugular vein was isolated and heparin (1,000 U/kg) was administered. The trachea was cannulated with a 16-gauge angiocath attached to a rodent ventilator (Harvard Apparatus, South Natick, MA) to set to provide adequate ventilation with room air. A midsternal thoracotomy was performed to expose the heart and cannulate the aorta. After rapid cannulation of the aorta, retrograde coronary perfusion with Krebs-Henseleit buffer (1.25 mM CaCl₂, 5.5 mM glucose, 112 mM NaCl, 25 mM NaHCO₃, 5 mM KCl, 1.2 mM MgSO₄, 1 mM K₂HPO₄, and 0.2 mM octanoic acid, bubbled with 95% O₂-5% CO₂, pH 7.4) was initiated in situ. Hearts were quickly excised from the chest and transferred to the Langendorff apparatus with warmed (37°C) Krebs-Henseleit buffer perfusion at 85 mmHg. A saline-filled latex balloon attached to a pressure transducer was inserted into the left ventricle for measurement of left ventricular (LV) contractile function. The hearts were positioned in a temperature-controlled (37.4°C) glass chamber. Coronary flow rates were continuously measured and monitored using an in-line electronic flowmeter (T206; Transonic Systems, Ithaca, NY).

The LV balloon volume was inflated at the beginning of the experiment to a LV end-diastolic pressure of 5 mmHg. LV pressure was continuously sampled at a frequency of 45 Hz and digitally processed using a heart performance analyzer (Digit-Med; Micro-Med, Louisville, KY). Continuous measures of LV function were derived by computer algorithm: LV systolic pressure, LV end-diastolic pressure, heart rate, LV contractility (dP/dt max and negative dP/dt max), developed pressure (systolic – diastolic pressure), and rate-pressure product (developed pressure × heart rate) were calculated.

Perfusion protocol. Isolated hearts underwent a 15-min period of equilibration perfusion followed by a 10-min baseline period of perfusion with 95% O₂-5% CO₂-bubbled perfusate. Hearts were subjected to 20 min of global ischemia, an ischemic duration previously noted to produce stunning without necrosis (22). Reperfusion was with different oxygen concentrations: group 1, 95% O₂-5% CO₂; group 2, 20% O₂-75% N₂-5% CO₂; or group 3, 95% N₂-5% CO₂. Oxygen percentage of the perfusate bubbled with 95% N₂-5% CO₂ was measured at the level of the aorta and was determined to contain 2% oxygen. The reduced oxygen concentrations in groups 2 and 3 were maintained for the first 5 min of reperfusion, after which the perfusion was changed to 95% O₂-5% CO₂ oxygenated perfusate.

In a second set of experiments, hearts were loaded with various blockers before ischemia. Hearts were perfused with either diphenyleneiodonium (DPI; 1 μM) to block NAD(P)H oxidase (24), allopurinol (1 mM) to block xanthine oxidase (16), or Tiron (10 mM), a superoxide scavenger and heavy metal chelator (28). After 20 min of global ischemia, hearts were reperfused with 95% N₂-5% CO₂-bubbled perfusate for the first 5 min and then changed to 95% O₂-5% CO₂. Effluent was collected from the heart at 20-s intervals for the first 10 min of reperfusion. Free radical concentration in the effluent was determined using EPR spectroscopy. At the end of 30 min of reperfusion, all hearts were freeze-clamped in liquid nitrogen. Hearts were lyophilized for 24 h, weighed to determine dry heart weight, and stored in a ~80°C freezer.

Myocardial tissue oxygen tension. Microcrystalline particulates of lithium octa-n-butoxyphthalocyanine (LiNc-BuO) measuring 5–10 μm were implanted in the LV wall of perfused isolated rat hearts to measure interstitial tissue PO₂ (19, 23). The LiNc-BuO probe yields a

Fig. 1. Myocardial reactive oxygen species (ROS) formation at reperfusion with different O₂ concentrations. The ROS were measured as 5,5-dimethyl-1-pyrrole-N-oxide (DMPO) adducts in the effluent by using spin-trapping electron paramagnetic resonance (EPR) spectroscopy. The DMPO adduct peaked in the first 2 min in all groups, and the cumulative DMPO adduct in the first 2 min was significantly elevated in the most hypoxic reperfusion group (2% O₂) compared with the 20 and 95% O₂ reperfusion groups (*P < 0.001 vs. 95% O₂ and 20% O₂; n = 4/group).

Fig. 2. EPR spectra of effluent of heart perfusate: preischemia (A), effluent collected 2 min after reperfusion with 40 mM DMPO, showing hydroxyl (DMPO-OH) adduct and alkyl adduct (B), simulation of DMPO-OH adduct (C), simulation of DMPO-alkyl adduct (D), and simulation of both DMPO-OH adduct and DMPO-alkyl adduct (C + D, compare with Fig. 4B). Microwave frequency, 9.786 GHz; microwave power, 10 mW; modulation amplitude, 1 G; scan time, 30 s; no. of scans, 10.
single sharp EPR line, the width of which is highly sensitive to oxygen tension. Decreased oxygen tension results in sharpening of the EPR spectrum, which yields a linear PO2 response from 0 to 760 Torr (14). The isolated perfused hearts were placed in the active volume of a reentrant resonator of an L-band (1.2 GHz) EPR spectrometer that was controlled using custom-designed software (14, 17). This gives continuous online measurement of localized myocardial oxygen tension throughout ischemia and reperfusion. This technique allows measurement of the absolute tissue PO2 values ranging from very low oxygen levels to supranormal physiological levels.

5,5-Dimethyl-1-pyrroline-N-oxide adduct at reperfusion. The concentration of oxygen free radicals at reperfusion was measured using the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (29) infused directly into the heart by using an infusion pump through a side arm. DMPO at 40 mM (final concentration) was infused for 20-s preischemia (control) and restarted with reperfusion. Effluent samples in 1-ml quantities were collected every 20 s up to 120 s and then at 1- to 2-min intervals up to 10 min of reperfusion and then immediately frozen in liquid nitrogen for EPR measurements. Samples were then analyzed using an EPR-300 X-band (9.7 GHz) spectrometer. Spectral simulations were performed to identify specific radicals. Quantification of the free radical signals was performed by comparing the double integral of the observed signal with that of a known concentration of a free radical standard in aqueous solution (29).

Aconitase. Tissue aconitase activity was measured at the end of 30 min of reperfusion. Tissue aconitase is inactivated by superoxide, and the amount of inhibition of aconitase activity correlates with superoxide production (9, 10). The assay was performed on homogenized tissue obtained from hearts freeze-clamped at the end of the reperfusion period. Absorbance was followed at 340 nm in Tris buffer, with 5 mM sodium citrate as a substrate, 0.6 mM MnCl2, 0.2 mM NADP, and 2 U of isocitrate dehydrogenase (10, 11).

Adenine nucleotide analysis. To determine tissue adenine nucleotide concentrations at the end of 30 min of reperfusion, hearts were freeze-clamped and lyophilized, and metabolites were extracted with 0.6 N perchloric acid. Extracts were neutralized with NaHCO3 and analyzed spectrophotometrically for ATP, phosphocreatine (PCr), and 2Uo fisocitrate dehydrogenase (10, 11).

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Fig. 3. Myocardial tissue aconitase activity in the reperfused heart. Aconitase activity (mU/mg dry heart wt, DHWt) was measured at 30 min of reperfusion after an initial 5 min of reperfusion with varied O2 concentrations. The aconitase activity was significantly depressed in the 2% O2 group (*P < 0.03, n = 6/group).

Fig. 4. Myocardial tissue oxygen tension (PO2) during global ischemia and early reperfusion. The tissue oxygenation was measured using EPR spectroscopy with an implanted oxygen-sensing probe. A rapid decline in myocardial tissue PO2 is noted with the onset of global ischemia. A significant depression in myocardial PO2 is indicated during the first 5 min of reperfusion with 2% O2 compared with 95% O2 (*P < 0.05, n = 4/group).

Fig. 5. Coronary flow during reperfusion of hearts perfused with 95, 20, and 2% O2 for the first 5 min of reperfusion and then switched to 95% O2. Coronary flow was calculated as a percentage of preischemic values (n = 4/group) and was similar among groups.

Fig. 6. Recovery of left ventricular contractility (dP/dtmax) during reperfusion with variable O2 concentrations. Groups were reperfused with 95, 20, or 2% O2-saturated perfusate for the first 5 min of reperfusion and then all changed to 95% oxygenated perfusate for the remainder of the reperfusion period. The recovery of dP/dtmax was significantly increased in the 95% O2 group (*P < 0.001 vs. 20% O2 and 2% O2; n = 6/group).
According to the figure, the rate-pressure product (RPP; heart rate × developed pressure) in groups reperfused with 95, 20, or 2% O₂-saturated perfusate for the first 5 min of reperfusion and then all changed to 95% oxygenated perfusate for the remainder of the reperfusion period. RPP was significantly increased in the 95% O₂ group (∗∗P < 0.001 vs. 20% O₂ and 2% O₂; n = 6/group).

In all three reperfusion groups, oxygen free radical formation, as measured by DMPO adduct, peaked in the first 2 min of reperfusion and was significantly higher in the 2% O₂ group compared with the 95 and 20% O₂ groups (Fig. 1). After the initial burst, the levels of DMPO adduct returned to near-basal levels in each group. The DMPO adduct consisted primarily of DMPO-OH and DMPO-R (from trapping of hydroxyl and carbon-based free radicals, respectively) (Fig. 2). In the same hearts, aconitase activity measured at the end of the 30-min reperfusion period was significantly depressed in the 2% O₂ group compared with the 95% O₂ group (Fig. 3).

Myocardial tissue oxygen tension preischemia was similar in all groups (Po₂ = 217 ± 5 Torr). With the onset of global ischemia, tissue oxygen tension rapidly declined to a mean of <30 Torr by 1 min of ischemia (P < 0.001 vs. baseline for all groups). At the end of the 20-min global ischemia period, tissue oxygen tension (in Torr) was 7.10 ± 2.45 in group 1, 8.70 ± 5.70 in group 2, and 7.94 ± 4.80 in group 3. With the onset of reperfusion, tissue oxygen tension was significantly higher during the initial 5 min in the 95% vs. 2% O₂ reperfusion group (Fig. 4). At 5 min of reperfusion, the perfusate was changed to 95% O₂-saturated perfusate in all groups. During the reperfusion period, the tissue Po₂ gradually increased, although it remained significantly depressed compared with preischemic baseline values. Reperfusion coronary flow was similar in all groups and recovered to ≈70–80% of baseline flow (Fig. 5). There was no difference in coronary flow between groups in the first 5 min of reperfusion.

Recovery of LV function during the 30-min reperfusion period was significantly depressed in all groups compared with preischemia. LV contractility, measured as dP/dt max, was significantly depressed during reperfusion in the 2% O₂ group compared with the 95% O₂ group (Fig. 6). Other measures of LV function, including rate-pressure product, were similarly depressed in the 2% O₂ group compared with the 95% O₂ group (Fig. 7). At the end of 30 min of reperfusion, tissue ATP, PCR, and phosphorylation potential were all significantly reduced in the 2 and 20% O₂ groups compared with the 95% O₂ group and nonischemic controls (Table 1). A significant increase in creatine kinase release was noted during reperfusion in the 2% O₂ hearts compared with 95% O₂ reperfused hearts (Table 2).

In hearts reperfused with low (2% O₂) oxygen for the first 5 min in the presence of ROS inhibitors, a significant reduction in DMPO adduct with DPI and Tiron was noted (Fig. 8). Allopurinol-treated hearts tended to have a reduction of DMPO adduct at 2 min of reperfusion, but over the first 2 min of reperfusion, DMPO adduct cumulatively was not different from 2% O₂ controls. Recovery of dP/dt max at the end of 30 min of reperfusion was significantly increased in hearts reperfused with Tiron and DPI (Fig. 9). In all groups, an inverse correlation was noted between the recovery of contractility (%recovery dP/dt max) in the first 30 min and ROS (DMPO adduct) concentration at the onset of reperfusion (R = −0.709, P < 0.05).

**DISCUSSION**

The present study demonstrates the key role of tissue oxygen tension in mediating the burst of ROS formation at reperfusion in the whole heart and the association of early ROS formation with initial depression of contractile function. In this study we noted significantly higher ROS production in the more hypoxic tissue, in contrast with higher myocardial tissue oxygen tension, which yielded lower ROS production. Using various inhibitors of key enzymes responsible for ROS, we noted that the source of ROS seems to be from multiple pathways including xanthine oxidase and NAD(P)H oxidase, because the ROS signal could only be partially blocked with xanthine oxidase or NAD(P)H oxidase inhibitors. Tiron, a superoxide

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**Table 1. Bioenergetic values after 30 min of reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ATP, μmol/gdw</th>
<th>PCR, μmol/gdw</th>
<th>Creatine, μmol/gdw</th>
<th>PCr, mM</th>
<th>Phosphorylation Potential, M⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemic controls</td>
<td>8</td>
<td>20.6±1.6</td>
<td>27.9±4.7</td>
<td>26.9±4.4</td>
<td>0.5±0.04</td>
<td>200±25</td>
</tr>
<tr>
<td>95% O₂</td>
<td>6</td>
<td>9.7±1.4</td>
<td>27.1±3.3</td>
<td>9.3±3.1</td>
<td>3.4±1.2</td>
<td>249±90</td>
</tr>
<tr>
<td>20% O₂</td>
<td>6</td>
<td>5.0±0.7†</td>
<td>15.6±2.3†</td>
<td>19.3±2.5</td>
<td>9.4±3.3</td>
<td>17±3†</td>
</tr>
<tr>
<td>2% O₂</td>
<td>6</td>
<td>4.2±0.7*</td>
<td>12.7±2.5*</td>
<td>17.0±3.7</td>
<td>6.1±1.6</td>
<td>33±16*</td>
</tr>
</tbody>
</table>

Bioenergetic values at the end of 30 min of reperfusion, following initial 5 min of controlled oxygen reperfusion and 25 min of reperfusion with 95% O₂ compared with concurrent nonischemic controls. ATP, phosphocreatine (PCR), and phosphorylation potential ([PCR]/[creatinine × PCr]) were all significantly lower in the 2% O₂ reperfusion group. *P < 0.05 vs. 95% O₂ and nonischemic controls. †P < 0.05 vs. 2% O₂ group.
scavenger, nearly completely blocked the DMPO adduct signal at reperfusion, suggesting that superoxide is the primary source of the radicals. In addition to scavenging superoxide directly, Tiron by virtue of its metal-chelating properties also may limit superoxide production through inhibition of the iron-mediated Fenton reaction. Modulating the ROS burst at reperfusion has functional significance, as indicated by the association of ROS production with the return of myocardial contractile function in the early reperfusion period. In all groups, there was an inverse relationship between radical production in the first 10 min, most of which occurred in the first 1–4 min, and the return of contractile function. This was most prominently seen with Tiron. Tiron, which had the greatest effect on suppression of the DMPO adduct signal, resulted in the greatest improvement in the recovery of contractile function. These data confirm our hypothesis that tissue oxygen tension is a primary determinant of the initial ROS generation at reperfusion. Using EPR spectroscopy, we measured tissue oxygen tension in the heart tissue, rather than vascular or perfusate PO2, to more reliably determine the oxygen available to the cells during initial reperfusion. Although tissue oxygen tension was low in all groups during reperfusion, we did confirm significant tissue oxygen tension differences in the first 5 min of controlled oxygen reperfusion, a time when the burst of ROS production peaked. This EPR method of tissue oxygen measurements recently was used in vivo in a mouse regional ischemia model to measure changes in tissue PO2 under conditions of ischemia and reperfusion (27). Myocardial tissue PO2 values at baseline were higher in our model because of the high PO2 of the perfusate relative to that of normal perfused blood; however, values during ischemia were very close to values measured during ischemia in the in vivo heart.

This work is consistent with earlier studies in vascular tissue in which DMPO-measured adduct could be blocked with superoxide dismutase but not with catalase, suggesting that the main source of ROS is superoxide (24). Although earlier work has identified xanthine oxidase in endothelial cells as the main source of superoxide (31), recent work has emphasized NAD(P)H oxidase as an important source of ROS, particularly in the vascular wall (12, 25). This NAD(P)H oxidase, also a producer of superoxide, is associated with the sarcolemmal membrane and is distinct from the phagocytic NAD(P)H oxidase (24). Our results in the whole heart under actual ischemia and reperfusion conditions are compatible with earlier embryonic chick cardiomyocyte work in which oxygen conditions were varied and ROS was measured using oxidant-sensitive probes. During ischemia, ROS production was greatest with the most hypoxic perfusion conditions (8). It was postulated that hypoxia increases the lifetime of reduced electron carriers such as ubiquinone, which facilitates increased superoxide production, allowing for increased ROS production. A key difference with that work and the present study is that ROS measured in the present study were generated at reperfusion and are in much higher quantities than ROS production during ischemia. Whereas low-level ROS production during ischemia may have important cardioprotective signaling functions, the large ROS burst at the onset of reperfusion, as demonstrated in this study, inhibits contractile recovery. Nevertheless, the same mechanisms that account for greater ROS formation under hypoxic conditions during ischemia may account for increased ROS formation under hypoxic conditions at reperfusion.

These studies do not allow us to specifically identify the compartment source of the ROS burst measured during early reperfusion. In the whole heart there are many potential

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**Table 2. Creatine kinase values in heart effluent**

<table>
<thead>
<tr>
<th>Reperfusion Group</th>
<th>n</th>
<th>5-min Reperfusion</th>
<th>25-min Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% O2</td>
<td>6</td>
<td>19.4±4.1</td>
<td>29.4±7.8</td>
</tr>
<tr>
<td>20% O2</td>
<td>6</td>
<td>25.4±5.3</td>
<td>65.1±8.9</td>
</tr>
<tr>
<td>2% O2</td>
<td>6</td>
<td>43.0±17.9</td>
<td>103.1±23.2*</td>
</tr>
</tbody>
</table>

Creatine kinase (IU/l; means ± SE) at 5 and 25 min of reperfusion, following 20 min of global ischemia. *P < 0.01 compared with 20% O2 and 95% O2 groups.
soures of ROS formation, including membrane sources of xanthine oxidase and NAD(P)H oxidase and intracellular sources, e.g., cytosolic and mitochondrial. The source of ROS in these studies appears to be of multiple origins, because the signal was only partially blocked by inhibition of xanthine oxidase with allopurinol and inhibition of NAD(P)H oxidase with DPI. This is in contrast to other studies in which the total inhibition of the DMPO-OH signal with DPI was noted in vascular tissue (24). The spin trap DMPO traps primarily extracellular radicals when infused during reperfusion and collected in the heart effluent. This measure of radical formation may in large part reflect radicals of vascular origin, as opposed to intracellular and particularly mitochondrial ROS generation, which is likely the primary source of cellular ROS. However, some measured ROS likely did come from intracellular sources, as indicated by Tiron’s ability to block the DMPO adduct signal. Tiron is a membrane-permeable scavenger of superoxide (28). Further evidence in support of mitochondrial ROS involvement was seen with differences in ATP and PCr levels between the 2 and 95% O2 reperfusion groups. Similar to aconitase, ATP and PCr levels were significantly depressed in the hypoxic reperfusion groups that had the largest ROS burst at reperfusion. The relative importance of various compartment-localized ROS formation, particularly in the mitochondria, remains unclear. Mitochondria-based ROS effects may differ from the ROS measured in this study and have unique effects at reperfusion. Characterization of ROS formation within the various extra- and intracellular compartments is needed before we can determine the overall effect of ROS formation with the onset of reperfusion in the heart. In this regard, future studies using transgenic models that over- or underexpress key ROS-generating systems will be insightful. In this study inhibitors were perfused into the heart before ischemia to be present in the tissues at the onset of reperfusion. It is possible that these inhibitors may have altered effective ROS formation during the global ischemia period, causing an unspecified effect on ROS formation at reperfusion.

This study focused on the first few minutes of reperfusion and the implications of ROS formation on early return of contractile function. This is a critical time period for successful resuscitation from cardiac arrest. Most of the current resuscitation failures from cardiac arrest occur within minutes. Although ~20% of cardiac arrests with current CPR and advanced life support methods do obtain restoration of spontaneous circulation, only ~25% of these patients ultimately survive. Early re-arrest and death following the initial successful reperfusion often occurs in the first minutes of reperfusion. This is likely the time period most impacted by the initial ROS generation of reperfusion, and thus measures that lessen this initial ROS burst are likely to improve early return of myocardial function and facilitate overall improved cardiac arrest survival. Future directions should include extrapolation to the whole animal model to determine whether controlled oxygen delivery during initial cardiac arrest resuscitation lessens ROS formation and improves recovery of cardiac function. This will allow evaluation of ROS formation and its effect on other key organs, including the brain.

In summary, we have demonstrated the importance of myocardial tissue oxygen tension during reperfusion and its role in modulating the burst of ROS at the onset of reperfusion. The significant new finding of this study is that the reperfusion burst of ROS in cardiac tissue is increased rather than decreased when initial oxygen is limited. Tissue hypoxia mediates an increase in the ROS burst at reperfusion and is associated with further impairment of LV functional recovery. The greatest increase in ROS formation was noted in the most hypoxic myocardial tissue at the time of reperfusion. This study offers further evidence in support of ROS-mediated inhibition of myocardial contractile recovery, as indicated by an inverse relationship of ROS formation and the return of contractile recovery after global ischemia of the heart.

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