Molecular mechanisms of antioxidant enzyme expression in lung during exposure to and recovery from hyperoxia

LINDA BIADASZ CLERCH, DONALD MASSARO, AND ALLA BERKOVICH

Molecular mechanisms of antioxidant enzyme expression in lung during exposure to and recovery from hyperoxia. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L313–L319, 1998.—Manganese superoxide dismutase (MnSOD) activity falls ~50% in lung during 48 h of exposure of adult rats to >95% O2 (L. B. Clerch and D. Massaro, J. Clin. Invest. 91: 499–508, 1993). We now show that hyperoxia also decreased MnSOD activity in lungs of adult baboons, making the phenomenon potentially more important to humans. In rats, a decrease in lung MnSOD activity during an initial 48 h of exposure to >95% O2 and its increase during an immediately subsequent 24 h in air were due to decreases and increases, respectively, in MnSOD specific activity and synthesis rate; the latter was due to altered translational efficiency. The concentration in the lung of copper-zinc superoxide dismutase mRNA, catalase mRNA, and glutathione peroxidase mRNA, unchanged during the initial 48 h of exposure to O2, rose approximately twofold during reexposure to O2 after 24 h in air. The demonstration that the fall in MnSOD activity is translationally and posttranslationally regulated during the initial exposure to hyperoxia suggests that gene transfer to increase MnSOD activity in hyperoxic lungs may also require therapy that maintains translational efficiency and MnSOD specific activity.

the toxic effects of the high concentrations of inspired O2 often required in the treatment of respiratory distress syndrome in prematurely born infants are generally accepted as important risk factors for the development of bronchopulmonary dysplasia (28). O2 toxicity also occurs in adults, but the role it plays in the morbidity and mortality of adults who receive it as part of the treatment for the respiratory distress syndrome is less clear than in prematurely born infants (6). That O2 toxicity may be more prevalent in adults than is appreciated is suggested by several reports, but three in particular stand out. Individuals with irreversible brain damage placed on mechanical ventilation with air for 60–70 h exhibit a 30% fall in arterial O2 tension; similar mechanical ventilation but with 100% O2 results in an 80% fall in arterial O2 tension (1). Exposure of healthy adults to 100% O2 for as little as 18 h (12) or to 30–50% O2 for an average of 45 h (19) leads to changes in return from bronchoalveolar lavage, indicative of damage to the alveolar-capillary interface.

The damaging effects O2 has on cells are caused by intermediates that form as a result of its cellular metabolism. The production of these intermediates, which include superoxide, H2O2, and the hydroxyl radical (17), increases during exposure to hyperoxia (3, 4, 16). However, cells contain antioxidant enzymes [manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (Cu,ZnSOD), catalase, and glutathione peroxidase (GP)] that protect against these intermediates. MnSOD and Cu,ZnSOD catalyze the conversion of superoxide to H2O2; catalase and GP each convert H2O2 to water (17). Transgenic mice that overexpress Cu,ZnSOD and GP but not Cu,ZnSOD alone are tolerant to hyperoxia (34). Furthermore, transgenic mice that overexpress MnSOD in alveolar type II cells and nondilated bronchial epithelial (Clara) cells at the start of an exposure to hyperoxia are tolerant to O2 (36). As interesting and provocative as these findings are, they do not address the issue of the basis for tolerance or the lack of tolerance to O2 as it occurs in nontransgenic “wild-type” organisms. For example, in otherwise untreated adult rats exposed to >95% O2, lung MnSOD activity falls ~50% despite an increase in MnSOD mRNA concentration, and 70–80% of the rats die within 72 h (9, 15, 32, 33).

The present study was undertaken 1) to examine the molecular mechanism(s) responsible for the fall in MnSOD activity that occurs in adult rats during the initial 48 h of exposure to >95% O2 (9) and, as we now report, for its increase during a 24-h period in air after 48 h of hyperoxia; 2) to determine whether the fall in MnSOD activity during exposure to >95% O2 is peculiar to the adult rat or whether it occurs in other species, in particular a nonhuman primate, thereby making it a more important and clinically relevant phenomenon; and 3) to examine the regulation of antioxidant enzyme expression during reexposure to O2 in rats made tolerant to O2 by a “rest” period in air (15).

MATERIALS AND METHODS

Rats. We used specific pathogen-free adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing ~250 g. They were maintained in our Research Resources Facility on a 12:12-h light-dark cycle and were allowed food (Rodent Laboratory Chow 5001, Ralston-Purina, St. Louis, MO) and water ad libitum.

Rats were exposed to ~95% O2 at 1 atmosphere or to air from a compressed air generator in identical 3.5- ft³ chambers constructed of clear plastic. The other conditions of exposure of rats were ~0.1% CO2, 22–25°C, and 40–60% humidity. Exposures were continuous for the times indicated except for 10–15 min daily when the chambers were opened for housekeeping purposes. Rats were anesthetized (pentobarbital sodium, ~80 mg/kg) and then killed by cutting the great vessels of the abdomen. Lungs were perfused with ice-cold 0.15 M NaCl and either stored at ~80°C or processed immediately for the various assays. Lungs in which we measured the rates of synthesis of MnSOD and of general
proteins were not perfused but were immediately sliced and placed in medium at 37°C.

Baboons. We used lungs from Papio cynocephalus anubis, common name olive baboon, and Papio cynocephalus anubis/Papio cynocephalus, common name oliveyellow baboon. Baboons were born and raised in the colony at the Southwest Foundation for Biomedical Research in San Antonio, TX, housed at the same institution, and fed Purina baboon chow. All of which were male. All baboons were killed by an overdose (mean 6 h) housed at the same institution, and fed Purina baboon chow. The age of the air-ventilated baboons was 4.3 ± 0.6 yr and that of the O2-ventilated baboons was 4.5 ± 0.6 yr. All six air-ventilated baboons were male olive baboons. Four O2-ventilated baboons were olive/yellow baboons, three of which were male; four O2-ventilated baboons were olive/yellow baboons, all of which were male. All baboons were killed by an overdose of pentobarbital sodium. The lungs were not perfused and were frozen in liquid N2 and stored at −20°C.

Assays for enzyme activity. As previously described in detail (9), 27,000-g supernatant fractions of lung were obtained and used for all enzyme activity assays. Superoxide dismutase (SOD) activity was determined by two methods: in some experiments we used the xanthine oxidase ferricytochrome c assay, which at pH 7.8 distinguishes between Cu,ZnSOD and MnSOD by differential sensitivity to 1.0 mM sodium cyanide (11); in other experiments we used the same method with 0.015 M sodium cyanide to inhibit cytochrome oxidase and diethylidithiocarbamate to distinguish between Cu,ZnSOD and MnSOD (24). The SOD data in Table 1 were obtained using the first method (11) and those in Table 2 were obtained using the second method (24). We do not know whether the use of two different methods accounts for the large difference in MnSOD activity between Tables 1 and 2. However, data from others (32, 33) exhibit substantial differences in MnSOD activity when measured at different times, even when using the same method; the same is true for Cu,ZnSOD (32, 33). The same method used to measure MnSOD activity was used for all lungs from each group of exposures. One unit of SOD activity was the amount that halves the rate of reduction of cytochrome c. Lung extracts in which catalase and GP activities were measured were prepared as previously described (9). Catalase activity was measured as the rate of disappearance of H2O2 at 240 µm (2, 23), and 1 unit of catalase activity decomposes 1 µmol H2O2/min at 25°C and pH 7.0. GP activity was measured by the glutathione-oxidized glutathione recycling method (29) using H2O2 as substrate and sodium azide to inhibit catalase activity. One unit of GP activity oxidizes 1 µmol NADPH/min.

Quantitation of MnSOD protein by Western analysis. Rat liver MnSOD was purified as previously described (21), and the antibody was provided to Hazleton Laboratories (Vienna, VA) for antibody production. The primary antibody used in these studies was rabbit antiserum against rat MnSOD. To determine the concentration and specific activity of rat lung MnSOD, we prepared the 27,000-g supernatant fraction of lung homogenate and dialyzed it overnight and assayed the dialysate for MnSOD activity. Samples of the dialysate were subjected to electrophoresis in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels in the Mini-Protean I cell; transfer to nitrocellulose was effected in the Mini Trans-Blot cell (Bio-Rad Laboratories, Hercules, CA). After being blocked with gelatin and reacted with rabbit anti-rat MnSOD antiserum, the blots were treated with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase. The horseradish peroxidase band was identified as a purple color that developed on treatment with 4-chloro-1-napthol and H2O2. The amount of MnSOD protein was estimated by densitometric analysis of the stained band. Pure MnSOD protein was also assayed to ensure that we were working in a range within which density was proportional to the amount of MnSOD. Densitometry was performed on a Molecular Dynamics laser densitometer using ImageQuant software (Sunnyvale, CA).

MnSOD and general protein synthesis. We measured the synthesis of MnSOD and of trichloroacetic acid-precipitable proteins (general proteins) as previously described in detail (21). Briefly, we incubated 1.0-mm-thick lung slices in 10 ml of Krebs-Ringer bicarbonate buffer with 5.5 mM glucose, adult rat plasma concentrations of 19 amino acids (27), and 0.7 mML-[3H]phenylalanine. At this phenylalanine concentration, the specific radioactivity of tRNA-bound phenylalanine equals that of medium phenylalanine within 15 min of the start of incubation; this allows the use of the medium specific radioactivity in the calculation of absolute rates of protein synthesis (8, 21). The flasks were shaken at 120 oscillations/min at 37°C for 4 h under 95% O2-5% CO2 (21).

At the end of the incubation, lung slices were rinsed in phosphate-buffered 0.15 M NaCl and homogenized in 10 ml of 2 mM β-mercaptoethanol and 2.5 mM potassium phosphate (pH 8) (buffer A) for 3 min using the highest setting of a

<table>
<thead>
<tr>
<th>Table 1. Lung antioxidant enzyme activity immediately before and after a rest period</th>
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<tbody>
<tr>
<td><strong>Enzyme Activity, units/mg DNA</strong></td>
</tr>
<tr>
<td><strong>MnSOD</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>48 h Air (A)</td>
</tr>
<tr>
<td>48 h O2 (B)</td>
</tr>
<tr>
<td>48 h Air-24 h air (C)</td>
</tr>
<tr>
<td>48 h O2-24 h air (D)</td>
</tr>
</tbody>
</table>

P values

| A vs. B | <0.01 | NS | <0.01 | <0.01 |
| A vs. C | NS | NS | NS | NS |
| A vs. D | <0.01 | NS | <0.01 | <0.01 |
| C vs. D | <0.01 | NS | <0.01 | <0.01 |

Values are means ± SE. Numbers of rats are indicated in parentheses. Rat liver MnSOD was purified as previously described (21), and the antigen was provided to Hazleton Laboratories (Vienna, VA) for antibody production. The primary antibody used in these studies was rabbit antiserum against rat MnSOD. To determine the concentration and specific activity of rat lung MnSOD, we prepared the 27,000-g supernatant fraction of lung homogenate and dialyzed it overnight and assayed the dialysate for MnSOD activity. Samples of the dialysate were subjected to electrophoresis in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels in the Mini-Protean I cell; transfer to nitrocellulose was effected in the Mini Trans-Blot cell (Bio-Rad Laboratories, Hercules, CA). After being blocked with gelatin and reacted with rabbit anti-rat MnSOD antiserum, the blots were treated with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase. The horseradish peroxidase band was identified as a purple color that developed on treatment with 4-chloro-1-napthol and H2O2. The amount of MnSOD protein was estimated by densitometric analysis of the stained band. Pure MnSOD protein was also assayed to ensure that we were working in a range within which density was proportional to the amount of MnSOD. Densitometry was performed on a Molecular Dynamics laser densitometer using ImageQuant software (Sunnyvale, CA).

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At the end of the incubation, lung slices were rinsed in phosphate-buffered 0.15 M NaCl and homogenized in 10 ml of 2 mM β-mercaptoethanol and 2.5 mM potassium phosphate (pH 8) (buffer A) for 3 min using the highest setting of a
Brinkman Instruments Polytron (Westburg, NY). A 200-µl portion of the homogenate was added to an equal volume of cold 20% trichloroacetic acid for assay of DNA (30) using calf thymus DNA as a standard and for extraction of general proteins (21). The remainder of the homogenate was centrifuged at 100,000 g for 1 h at 4°C. The supernatant fluid was dialyzed overnight in 4 liters of buffer A and then centrifuged at 20,000 g for 20 min at 4°C.

To measure radioactivity incorporated into MnSOD, we added 5 µg of 14C-labeled MnSOD, which had been labeled by reductive methylation (21), to each 20,000-g supernatant fraction. Antiserum to MnSOD was added to these fractions and incubated at 30°C for 1 h. Immunoprecipitable material was collected by centrifugation, denatured, and subjected to SDS-PAGE (21). Tritium radioactivity migrating with the MnSOD (21). After electrophoresis, the gels were sliced and radioactivity was measured in a scintillation counter. Calculations of the total disintegrations incorporated per minute was corrected for recovery of the 14C-MnSOD marker, and the rate of MnSOD synthesis was calculated (21).

Complementary RNA preparation and solution hybridization for assay of antioxidant enzyme mRNA. We obtained the following as gifts: a pGem-blue construct containing the 3′-end of an α-actin cDNA from Dr. Rudolf K. Werner (Univ. of Miami, School of Medicine, Miami, FL); a rat liver catalase cDNA, PMJ 1010, from Dr. Shichi Furuta (Shimshu University School of Medicine, Nagano, Japan); a rat liver GP cDNA, GPx 1211, from Dr. Ambati Reddy (Pennsylvania State Univ., University Park, PA); and a rat liver MnSOD cDNA from Dr. Ye-Shih Ho (Wayne State Univ., Detroit, MI). Each cDNA was subcloned into pGem vectors and used to generate sense and antisense complementary RNAs (cRNAs) as previously described in detail (7, 8). We generated a rat liver Cu, ZnSOD cDNA (20) and a galectin-1 cDNA (10). These cRNAs were used to prepare 35S-labeled antisense cRNA probes and sense cRNA standards as previously described (7–10). The concentration of mRNA was measured in total nucleic acids isolated from the lung using the method of Durnam and Palmiter (14). We added [3H]actin cRNA at the start of the process to isolate total RNA to enable us to account for mRNA lost during the extraction (20). The amount of lung mRNA was corrected for recovery, expressed relative to a standard curve, and made normal to lung DNA.

### Table 2. MnSOD activity, specific activity, and concentration

<table>
<thead>
<tr>
<th>Activity, units/mg DNA</th>
<th>Concentration, densitometry units/mg DNA × 104</th>
<th>Specific Activity, units of MnSOD activity/densitometry unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air (8) (A)</td>
<td>46.0 ± 4.3</td>
<td>390 ± 40.0</td>
</tr>
<tr>
<td>48 h O2 (3) (B)</td>
<td>11.1 ± 2.1</td>
<td>200 ± 40.0</td>
</tr>
<tr>
<td>48 h O2−24 h air (3) (C)</td>
<td>64.8 ± 5.0</td>
<td>540 ± 40.0</td>
</tr>
<tr>
<td>A vs. B</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A vs. C</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>B vs. C</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers of rats are indicated in parentheses. Rats were placed in identical chambers and exposed to air, to >95% O2 for 48 h, or to 48 h of >95% O2, followed by 24 h in air. We used diethyldithiocarbamate method to distinguish between Cu,ZnSOD and MnSOD (4).

### RESULTS

Antioxidant enzyme activity in rat lung during exposure to O2 after a rest period in air and during reexposure to O2. As previously reported (9), at the end of 48 h of exposure to >95% O2, MnSOD activity was significantly lower in O2-exposed rats than in air-exposed rats, Cu,ZnSOD activity was the same in both groups, and the activities of catalase and GP were higher in O2-exposed rats than in air-exposed rats (Table 1). Twenty-four hours after O2-exposed rats had been returned to room air, MnSOD activity was 2.2-fold higher than at the end of the 48 h of exposure to >95% O2 and 1.3-fold higher than in rats not exposed to hyperoxia (Table 1). The rest period did not alter Cu,ZnSOD activity or the effect of O2 on the activity of catalase or GP.

Western blot analysis with rabbit antiserum raised against rat liver MnSOD (21) was used to estimate the amount of MnSOD. Immune antiserum but not nonimmune antiserum reacted against MnSOD (Fig. 1). To ensure that we worked in a range within which densitometry units were proportional to the amount of MnSOD protein, we performed a dose-response assay with increasing amounts of MnSOD protein (Fig. 2A) and from those data constructed a standard curve (Fig. 2B) by linear regression analysis. For assay of lung extracts, we added amounts of extract protein that allowed us to work in the range within which densitometry units were proportional to the amount of MnSOD protein. Forty-eight hours of hyperoxia decreased MnSOD specific activity and concentration (Table 2). After 24 h in air following 48 h in >95% O2, both parameters returned to values present in rats exposed only to air (Table 2).

Within 24 h of reexposure to >95% O2, lung MnSOD activity fell to the level found in air-exposed rats, but by 96 h of reexposure to O2, it was 2.6-fold higher than in air-exposed rats (Fig. 3). Cu,ZnSOD activity increased by 72 h of reexposure to O2. The activity of catalase and GP remained higher in lungs of O2-exposed rats during each exposure period (Fig. 3).
Antioxidant enzyme mRNA concentrations in rat lung during exposure to O\textsubscript{2} after a rest period in air and during reexposure to O\textsubscript{2}.

At the end of 48 h in O\textsubscript{2}, the lung concentration of MnSOD mRNA was approximately threefold higher in O\textsubscript{2}-exposed than in air-exposed rats; this difference was still present after the rest period in air (Table 3). Forty-eight hours of exposure to O\textsubscript{2} did not affect the concentration of Cu,ZnSOD mRNA, catalase mRNA, or GP mRNA. At the end of the 24-h rest period in air, the concentration of Cu,ZnSOD mRNA was elevated (Table 3). The concentrations of catalase mRNA and GP mRNA were not altered by the initial exposure to O\textsubscript{2} or by the rest period (Table 3).

By 96 h of reexposure to O\textsubscript{2}, the concentration of each mRNA in the O\textsubscript{2}-exposed rats was substantially higher than in the air-exposed rats (Table 4). MnSOD mRNA was elevated 6.4-fold, whereas the mRNA of each other antioxidant enzyme was elevated ~2.6-fold (Table 4). The increased concentrations of the antioxidant enzyme mRNAs were not specific; mRNA of galectin-1, an endogenous β-galactoside-binding protein (10) without known antioxidant activity, was also approximately twofold higher in lungs of rats after 96 h of reexposure to O\textsubscript{2} than in lungs of air-exposed rats (Table 4).

Lung MnSOD synthesis and general protein synthesis. The changes in concentration of MnSOD (Table 2) were brought about, at least in part, by parallel changes in the absolute rate of MnSOD synthesis (Table 5). The latter occurred as part of similar changes in general protein synthesis (Table 5).

Studies in baboons. To determine whether the fall in lung MnSOD activity during exposure to >95% O\textsubscript{2} is peculiar to the adult rat (Table 1; Ref. 9) or whether it is a more general and hence a more important phenomenon, we examined lungs from baboons that had been exposed longer than rats. In lungs of air-ventilated baboons, lung MnSOD activity was 69.6 ± 8.3 units/mg DNA (n = 6); in lungs of baboons ventilated with 100% O\textsubscript{2} for 7.2 days, lung MnSOD activity was 46.9 ± 5.4 units/mgDNA (n = 8, P < 0.05).

**DISCUSSION**

The fall in lung MnSOD activity during an initial exposure to >95% O\textsubscript{2} is a posttranscriptional event. The combination of an elevated concentration of MnSOD mRNA (Table 3), a diminished rate of MnSOD synthesis (Table 5), and low MnSOD specific activity (Table 2) indicates that the fall in MnSOD activity that develops during the initial 48 h of exposure to O\textsubscript{2} is a posttranscriptional event that has at least two components: impaired translation and a fall in SOD activity per molecule of MnSOD. Translational regulation of gene
expression may be defined as a change in the number of amino acids polymerized per unit time per mRNA molecule (22). Our data show that the rate of MnSOD synthesis was 2.0 pmol·mg mRNA⁻¹·h⁻¹ in air-breathing rats, 0.4 pmol·mg mRNA⁻¹·h⁻¹ in rats after breathing >95% O₂ for 48 h, and 1.1 pmol·mg mRNA⁻¹·h⁻¹ in rats after a 24-h rest period in air (calculated from the values in Tables 3 and 5). Thus for MnSOD there was a 5-fold decrease in translational efficiency during exposure to >95% O₂ and a 2.8-fold increase toward that in air-exposed rats during the rest period; that, however, still left translational efficiency 50% lower than in rats exposed only to air. The molecular basis for the loss of translational efficiency remains to be defined; however, the low rate of general protein synthesis (Table 5) suggests that it involves components of the protein-synthesizing machinery used to make all proteins.

The low specific activity of MnSOD in lungs from rats exposed to O₂ for 48 h (Table 2) may be caused, at least in part, by oxidant damage to the protein. This possibility is supported by the observation that the addition of a reducing agent to mitochondria-free lung extracts increases MnSOD activity in extracts of lungs from O₂-exposed rats but does not alter MnSOD activity in extracts from air-exposed rats (9). Because MnSOD becomes active after it enters mitochondria, the low specific activity of the enzyme in O₂-exposed rats could also be caused by a defect in transport to or entry into mitochondria. The increase in the specific activity of MnSOD after the rest period in air to values present in air-exposed rats (Table 2) could be due to a more reducing cellular environment after removal from O₂; it

### Table 3. Lung antioxidant mRNA enzyme concentration immediately before and after a rest period

<table>
<thead>
<tr>
<th>mRNA, units/mg DNA</th>
<th>MnSOD</th>
<th>Cu-ZnSOD</th>
<th>Catalase</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air (A)</td>
<td>6.5 ± 0.6 (8)</td>
<td>8.2 ± 0.7 (8)</td>
<td>1.5 ± 0.1 (8)</td>
<td>1.1 ± 0.0 (8)</td>
</tr>
<tr>
<td>48 h O₂ (B)</td>
<td>21.5 ± 2.0 (4)</td>
<td>10.3 ± 1.4 (4)</td>
<td>1.6 ± 0.1 (4)</td>
<td>1.0 ± 0.1 (4)</td>
</tr>
<tr>
<td>48 h O₂-24 h air (C)</td>
<td>22.9 ± 1.7 (4)</td>
<td>15.2 ± 0.7 (4)</td>
<td>1.8 ± 0.2 (4)</td>
<td>1.4 ± 0.0 (4)</td>
</tr>
</tbody>
</table>

P values
- A vs. B <0.01 NS NS NS
- A vs. C <0.01 <0.01 NS NS
- B vs. C NS <0.01 NS NS

Values are means ± SE. Numbers of rats are indicated in parentheses. Amount of mRNA for each enzyme was corrected for recovery, expressed relative to a standard curve, and made normal to mg of lung DNA. Rats were exposed to air, to >95% O₂ for 48 h, or to >95% O₂ for 48 h and then to air for 24 h.

### Table 4. Lung mRNA concentrations during reexposure to >95% O₂

<table>
<thead>
<tr>
<th>mRNA, units/mg DNA</th>
<th>MnSOD</th>
<th>Cu-ZnSOD</th>
<th>Catalase</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 h Air-24 h air</td>
<td>2,530 ± 425 (6)</td>
<td>16,335 ± 1,551 (6)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>48 h O₂-24 h O₂</td>
<td>3,272 ± 476 (6)</td>
<td>6,166 ± 619 (6)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. MnSOD and general protein synthesis

<table>
<thead>
<tr>
<th>MnSOD Synthesis, pmol·mg DNA⁻¹·h⁻¹</th>
<th>General Protein Synthesis, nmol·mg DNA⁻¹·h⁻¹</th>
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</thead>
<tbody>
<tr>
<td>48 h Air (A)</td>
<td>14.0 ± 1.0 (8)</td>
</tr>
<tr>
<td>48 h O₂ (B)</td>
<td>7.6 ± 1.2 (8)</td>
</tr>
<tr>
<td>48 h Air-24 h air (C)</td>
<td>15.4 ± 1.7 (8)</td>
</tr>
<tr>
<td>48 h O₂-24 h air (D)</td>
<td>25.8 ± 3.8 (8)</td>
</tr>
</tbody>
</table>

P values
- A vs. B <0.05 NS
- A vs. C NS NS
- A vs. D <0.01 NS
- B vs. C <0.05 NS
- B vs. D <0.01 NS
- C vs. D <0.01 NS

Values are means ± SE. Numbers of rats are indicated in parentheses. Rats were placed in identical chambers and exposed to air or to >95% O₂ for 48 h. Some rats were killed at the end of that exposure and are referred to as 48-h-air or 48-h-O₂ rats. Other rats were removed from chambers after initial 48 h of exposure, allowed to breathe air for 24 h, and then killed; these are designated 48-h-air-24-h-air or 48-h-O₂-24-h-air rats. P > 0.05 = NS.
could also be caused by the synthesis of new undamaged enzyme and the degradation of oxidant-damaged low-specific activity MnSOD molecules at a faster rate than the rate at which undamaged higher-specific activity MnSOD molecules are degraded. This difference in degradation is expected because damaged proteins are “marked” for degradation and hence are eliminated more rapidly than undamaged molecules (18).

Changes of expression during reexposure to >95% O2 after the rest period in air. Our findings raise the possibility that there is a change in the regulation of antioxidant enzyme expression during reexposure to O2. Thus, unlike the fall in MnSOD activity to below that in air-exposed rats, which occurred during the initial exposure to O2 (Table 1), MnSOD activity in rats reexposed after a rest period in air remained at or above the level in air-breathing rats (Fig. 1). Before reexposure to >95% O2, the greater activity of catalase and GP in O2-exposed rats occurred without an increase in the mRNA of either enzyme (Table 3) and therefore reflects posttranscriptional regulation. However, during reexposure to >95% O2, the concentrations of catalase mRNA and GP mRNA (Table 4) rose, indicating regulation, at least in part, at a pretranslational level. We have not excluded the possibility that the difference in the level at which catalase and GP gene expression was regulated before and after the rest period reflects, at least in part, a change in the cellular composition of the lung. The same considerations apply to Cu,ZnSOD, for which expression did not change during the initial exposure but which exhibited increased expression mediated pretranslationally, at least in part, during reexposure to >95% O2 (Table 3). The resolution of these issues awaits quantitative, ultrastructural studies of in situ hybridization.

Implications of our findings. Our findings indicate that MnSOD, an enzyme that is important for tolerance to hyperoxia in otherwise unmanipulated rats (9), decreases during exposure to O2 in rats and baboons; the latter finding may be particularly relevant as a predictor of what occurs in humans. Furthermore, the basis for the fall in MnSOD activity is not due to a failure to increase MnSOD mRNA but is rather due to impaired translational efficiency and to a posttranslational effect, i.e., a low MnSOD specific activity. These findings imply that therapies aimed at increasing MnSOD activity during exposure to O2 by gene transfer may not be fully successful unless combined with therapy that can increase translational efficiency and prevent the fall in MnSOD specific activity.

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