O₂ regulates surfactant protein A mRNA transcription and stability in human fetal lung in vitro

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O₂ regulates surfactant protein A mRNA transcription and stability in human fetal lung in vitro. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L343–L350, 1998.—The effect of O₂ on surfactant protein (SP) A mRNA transcription and half-life was determined in midterm human fetal lung tissue cultured in either 20 (control) or 70% O₂. Incubation of tissues in 70% O₂ resulted in a 133% increase in SP-A mRNA transcription rate compared with control tissues. The SP-A mRNA half-life was increased by 54% in lung tissues cultured in 70% O₂ vs. control tissues. Western blot analysis indicated a threefold increase in SP-A in the 70% O₂ condition, demonstrating that O₂ regulation of SP-A mRNA levels results in corresponding changes in SP-A levels. Primer extension assays were performed to determine whether the observed increase in SP-A mRNA levels is secondary to the preferential expression of one of the human SP-A genes, SP-A1 or SP-A2. Transcripts of both the SP-A1 and SP-A2 genes were increased ~100% in tissues maintained in 70% O₂ compared with control tissues. These data demonstrate that O₂ regulates human SP-A mRNA levels by both transcriptional and posttranscriptional mechanisms. Furthermore, because there is no differential effect of O₂ on the expression of SP-A1 vs. SP-A2 mRNA, the properties of these genes that mediate regulation by O₂ must be conserved between the two genes.

Oxygen; messenger ribonucleic acid

O₂ tension and/or active O₂ species may regulate a number of processes, including aging, the promotion of tumorigenicity, vascular reactivity, erythropoiesis, and protection against hyperoxic damage, by the induction of antioxidant enzymes (11). Acarregui et al. (2) previously reported that O₂ tension regulates the differentiation of human fetal lung tissue in an explant model of lung development. Recently, the role of O₂ as a regulator of pulmonary surfactant-associated protein (SP) gene expression has been described in a number of systems. Horowitz et al. (16) demonstrated increased SP-A mRNA levels in the lungs of adult rabbits exposed to 100% O₂. SP-A levels have been demonstrated to be increased in the lungs of adult male rats exposed to 72 h of 85% O₂ (27), and mRNA levels for SP-A, SP-B, and SP-C have been demonstrated to be increased in the lungs of adult rats exposed to hyperoxia for 5 days, with the greatest increase in SP-A mRNA levels (28). SP mRNA levels are also increased in the lungs of preterm baboons ventilated under hyperoxic conditions (26). Acarregui et al. (1) previously demonstrated significant O₂-mediated increases in steady-state levels of SP-A mRNA in human fetal lung explants containing differentiated type II cells. This increase in SP-A mRNA was observed in tissues incubated in atmospheres of 70 and 95% O₂ compared with human fetal lung explants incubated in 20% O₂ and was not associated with changes in tissue morphology.

SP-A is a 35,000-molecular-weight glycoprotein in which the developmental expression is associated with increased glycerophospholipid production in the developing lung. SP-A binds strongly to surfactant phospholipids in the presence of calcium, contributes to the surface tension-lowering property of surfactant, and mediates the endocytosis and reutilization of surfactant phospholipids via binding to specific high-affinity receptors on the surface of type II pneumocytes (25). There are two human SP-A genes, designated SP-A1 and SP-A2, that are 94% identical at the nucleotide level and 96% identical in their amino acid sequences (23). The human SP-A1 and SP-A2 genes are differentially regulated by adenosine 3',5'-cyclic monophosphate (cAMP) and glucocorticoids in human fetal lung explants (24).

As indicated above, the regulation of SP-A gene expression by O₂ has been amply demonstrated. However, the mechanisms whereby O₂ regulates the levels of SP-A are unknown. SP-A is the most abundant SP, and because SP-A is known to be important for surfactant function, further information regarding the mechanisms governing SP-A production is of interest to those studying lung development. Furthermore, information regarding the molecular regulation of SP-A production by O₂ may shed light on the regulation of other gene products that are also modulated by O₂ tension. Therefore, the goal of the present study was to determine whether O₂ regulates SP-A mRNA steady-state levels at a transcriptional or posttranscriptional level. Transcription elongation assays were employed to compare the rate of transcription for SP-A mRNA in human fetal lung explants incubated in room air (20% O₂) vs. high-O₂ (70%) conditions. The effect of O₂ on the stability of the mRNA for SP-A was evaluated by determining the half-life of SP-A mRNA from human fetal lung tissues incubated at the two different O₂ concentrations. Finally, we compared the effect of O₂ on the expression of the SP-A1 and SP-A2 genes to discern whether the effects of O₂ on the steady-state levels of SP-A mRNA were due to selective induction of either or both SP-A genes in human lung. O₂ concentrations of 20 and 70% were employed in these studies because 70% O₂ is the lowest O₂ concentration that Acarregui et al. (1) previously found to cause a statistically significant increase in SP-A mRNA levels compared with that in human fetal lung explants maintained in 20% O₂.
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O2 AND SP-A IN HUMAN FETAL LUNG IN VITRO

MATERIALS AND METHODS

Organ culture. Lung tissues were obtained from human abortuses of 16- to 19-wk gestation from local sources or from Advanced Biosciences Resources (Alameda, CA) as approved by the University of Iowa Human Subjects Review Committee. Lung tissue from a single fetus was employed for each experiment performed, with the exception of the mRNA stability assays that required lung tissues from two fetuses for each experiment. Fetal lung tissues were prepared and maintained in serum-free Waymouth's MB752/1 medium (Gibco BRL, Life Technologies, Grand Island, NY) as previously described (32). The lung explants were cultured in a humidified 37°C incubator in 5% CO2-95% air (20% O2). After 4 days of incubation, the tissues were either maintained in the 37°C incubator in 5% CO2-20% O2 or moved into a modular incubator (Billups-Rothenberg, Del Mar, CA) and flushed with a gas mixture of 70% O2-5% CO2-25% N2 for up to an additional 36 h of incubation. The modular incubator was equilibrated daily with the 70% O2-containing gas as described previously (1), and the culture medium was changed every 24 h. The gaseous atmospheres were reestablished after each time point at which tissues were harvested. Human fetal lung explants used for the mRNA half-life determinations were harvested after 4 days in culture (time 0) and after a subsequent 4, 8, 12, 24, or 36 h of incubation in 20 or 70% O2 in the presence of actinomycin D (5 µg/ml) or actinomycin D and cycloheximide (2.5 µg/ml). Tissues used for transcription elongation assays were harvested after 24 h (5 days total) in 20 or 70% O2 and processed immediately as described in Isolation of nuclei and transcription elongation assay. The purpose of maintaining all tissues for mRNA stability and transcription elongation studies in 20% O2 for the first 4 days was to induce type II cell differentiation and to prevent any possible effect that different O2 concentrations might have on the rate of type II cell differentiation. To allow for an increased accumulation of SP-A and SP-A mRNA and thereby maximize the differences observed between conditions, the tissues processed for Western blotting and primer extension assays were maintained for 4 days in the 37°C incubator in 5% CO2 and either 20 or 70% O2 and were then harvested. These tissues and those from which total RNA was isolated were harvested and then immediately frozen in liquid N2 and stored at -70°C.

Immunoblot analysis. Midtrimester human fetal lung tissues that had been maintained in explant culture for 4 days were homogenized in ice-cold water containing phenylmethylsulfonyl fluoride (1 mM). Twenty micrograms of protein were separated by electrophoresis on a 10% polyacrylamide gel with a tris(hydroxymethyl)aminomethane (Tris)-glycine-sodium dodecyl sulfate (SDS) buffer and then transferred to Immobilon membranes (Millipore, Bedford, MA) by electrophoresis. The membranes were blocked overnight at 4°C, and SP-A was detected after incubation of the blots with guinea pig anti-human SP-A anti-serum as previously described (12). RNA isolation and Northern blot analysis for determinations of mRNA stability. Total RNA was isolated from frozen tissues by the method of Chomczynski and Sacchi (8). Northern blot analysis of RNA from human fetal lung explants was performed as previously described (1). For the mRNA half-life determinations, 10 µg of total RNA from each sample harvested at the different time points were separated on a 1.2% agarose-formaldehyde gel and then transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH) by vacuum blotting (Bio-Rad Laboratories, Hercules, CA) for Northern blot analysis of SP-A mRNA. After RNA was cross-linked to the membranes, the membranes were prehybridized at 42°C for 6-12 h and then hybridized at 42°C overnight in hybridization buffer containing 1 x 106 counts/min·ml-1 of 32p-labeled cDNA specific for human SP-A mRNA (the human SP-A cDNA was a kind gift from Dr. Jeffrey A. Whitsett, University of Cincinnati, OH) (13) and glyceraldehyde-3-phosphate dehydrogenase (GAPD) mRNA (American Type Culture Collection, Rockville, MD) or 18S rRNA. After hybridization, the blots were washed (5 x SSC; Bio-Rad Laboratories) with an intensifier screen at -70°C for 24-72 h. After the blots were hybridized to cDNAs for SP-A mRNA and GAPD mRNA, they were stripped and reprobed with cDNA for 18S rRNA. Autoradiograms of the hybridized blots were quantified by scanning densitometry. Values were corrected for RNA loading and transfer errors by adjustment to the relative amounts of 18S rRNA as determined by optical scanning of autoradiographs with an Ambis 4000 optical-imaging system (Ambis, San Diego, CA). 18S rRNA and GAPD mRNA levels were unaffected by O2 concentration.

Isolation of nuclei and transcription elongation assay. Isolation of nuclei and transcription elongation assays were performed by methods modified from those described by Boggaram and Mendelson (3). Explants that had been in culture for 4 days in 20% O2 and then for an additional 24 h in 70% O2 were homogenized with a Dounce homogenizer, and the nuclei were isolated by centrifugation through 1.3 M sucrose at 10,000 g for 10 min at 4°C. For transcription elongation assays, 2 x 107 nuclei were suspended in a total volume of 200 µl of reaction buffer and incubated in the presence of 0.4 mM each ATP, CTP, and GTP, 100 µCi of [α-32P]UTP (3,000 Ci/mmol), and 5 U of ribonuclease (RNase) inhibitor at 37°C for 20 min. The nuclei were then digested with 100 units of RNase-free deoxyribonuclease (DNase) I for 20 min at 37°C, followed by proteinase K (100 µg/ml) digestion in 15 mM EDTA, 0.1% SDS, and 30 µg of yeast tRNA for 30 min at 37°C. The labeled RNA was extracted with phenol-chloroform-isomyl alcohol (24:25:1) and then precipitated with ethanol. The specific activity of the RNA was determined by scintillation spectrometry.

Unlabeled cDNA for SP-A and human β-actin in Bluescript plasmids were linearized by restriction enzyme digest, denatured in 0.3 M NaOH, and applied to a Nytran membrane (Schleicher & Schuell, Keene, NH) by vacuum blotting. Linearized Bluescript plasmid DNA without a cDNA insert was applied to the membrane as a negative (background) control. After baking for 2 h at 80°C and prehybridizing at 42°C overnight, the membranes were incubated in hybridization buffer containing 2 x 106 counts·min-1·ml-1 of radiolabeled RNA at 42°C for 4 days. After hybridization, the filters were washed twice in 0.2 x saline-sodium citrate (SSC; 1 x SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0)-0.1% SDS at 50°C for 1 h and in 2 x SSC at room temperature for 15 min and then incubated with 10 µg/ml of RNase A and 1 µg/ml of RNase T for 30 min at 37°C. After two more washes in 2 x SSC at room temperature for 15 min, the filters were dried and exposed to Kodak XAR autoradiographic film at -70°C for 2-7 days. The autoradiographs were quantified by scanning densitometry. The assay was performed three times with nuclei from different cultured fetal lung explants from an individual fetus each time. Transcriptional levels for SP-A and β-actin mRNA were compared between O2 conditions.

Primer extension analysis. An oligonucleotide primer (5'-GGGGATACCCAGGCTTCAACACAAGC-3'), complementary to nucleotides 1117-1144 of human SP-A1 (23, 33) and nucleotides 1144-1171 of human SP-A2 (23, 19), was labeled at its 5'-end with [γ-32P]ATP (5,000 Ci/mmol; Amersham, Bedford, MA) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA) as previously described (30). Primer
extension was carried out with a modification of a standard protocol (30). Five micrograms of total RNA from human fetal lung tissues that had been incubated for 4 days in either the 20 or 70% O2 condition were incubated with 0.2 pmol of the radiolabeled primer for 10 min at 70°C and then cooled on ice for 10 min. The radiolabeled primer was then extended with 200 units of Superscript II reverse transcriptase (Gibco BRL, Life Technologies, Grand Island, NY) in 20 µl of buffer (50 mM Tris–HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2, 1 mM each dATP, dGTP, dCTP, and dTTP, 50 µg/ml of actinomycin D) and incubated at 42°C for 1 h. After the reaction was stopped with 1 µl of 0.5 M EDTA, pH 8.0, and the RNA template was digested with 1 µl of 5 µg/ml DNase-free RNase, the radiolabeled DNA transcripts were extracted, precipitated with ethanol, and then resuspended in 4 µl of 10 mM Tris–HCl (pH 7.6)-1 mM EDTA buffer. Six microliters of loading buffer (80% formamide, 10 mM EDTA, pH 8.0, 1 mg/ml of xylene cyanol, and 1 mg/ml of bromphenol blue) were added to each sample. The samples were denatured at 95°C for 2 min, placed on ice for 5 min, and then loaded on a 6% polyacrylamide-7 M urea sequencing gel. After electrophoresis at 58 W, the gel was transferred to filter paper, dried, and exposed to Kodak XAR film with an intensifier screen (Lighting Plus, DuPont, Wilmington, DE) at 270°C. The band densities were determined by scanning densitometry. Each experiment was performed five times. The densitometric values for the bands in each experiment were normalized to the control condition for that experiment. The change in band densitometric value compared with that for the control condition was used to calculate the percent increase or decrease in the relative abundance of the mRNA transcript the band represented. The mean ± SE was calculated for each condition. This method for comparing the relative abundance of each transcript for SP-A1 and SP-A2 has been recently validated (21).

RESULTS

Effect of O2 on immunoreactive SP-A in human fetal lung. To maximize the observed differences in O2-induced SP-A, midtrimester human fetal lung was maintained in explant culture in either 20 or 70% O2 for 4 days and then harvested. Immunoblotting revealed a threefold increase (range 1.3- to 6.0-fold in four separate experiments) in immunoreactive SP-A in tissues maintained in 70% O2 versus tissues incubated in 20% O2 (Fig. 1). These data corroborate the O2-induced increases in SP-A mRNA in human fetal lung in vitro that we previously observed by demonstrating that the levels of the translation product are also increased by O2.

Effect of O2 on transcriptional rate of SP-A in human fetal lung. To determine whether the rate of gene transcription for SP-A is modulated by O2, we cultured human fetal lung explants for 4 days in 20% O2 and then continued to maintain the tissues in 20% O2 or changed the tissues to an atmosphere of 70% O2 (21). Tissues were then maintained for an additional 24 h in standard 5% CO2-20% O2 conditions or moved to an atmosphere of 5% CO2-70% O2-25% N2. Nuclei were isolated from tissues, and transcription elongation assays were performed to determine rate of transcription of SP-A mRNA in 20 and 70% O2 conditions. Relative rates of transcription were determined by scanning densitometry. A: values for 20% O2 condition were normalized to one. Data are densitometric determinations corrected for loading errors and background by β-actin and Bluescript vector (BKSK) values. Values are means ± SE for 3 separate experiments, each using nuclei collected from lung explants from a different fetus. *P < 0.01 vs. 20% O2 condition. B: representative autoradiogram of a transcription elongation assay.
O2 was 2.33 ± 0.37 densometric units compared with 1.00 ± 0.48 densometric units in tissues maintained in a 20% O2 atmosphere (Fig. 2). This statistically significant 2.3-fold increase in the transcription rate of SP-A mRNA in human fetal lung tissues demonstrates transcriptional control of SP-A gene expression by O2.

Effect of O2 on SP-A mRNA stability. To explore the effect of O2 on SP-A mRNA stability, we incubated human fetal lung tissues for 4 days in 20% O2 and then either maintained the tissues for up to an additional 36 h of incubation in 20% O2, or moved the tissue explants into a modular incubator containing a gas mixture of 70% O2-5% CO2 for up to an additional 36 h of incubation. Explants were harvested at timed intervals starting at the end of 4 days of culture, which was time 0, or after 4, 8, 12, 24, or 36 h of incubation in either 20 or 70% O2 in the presence of actinomycin D or actinomycin D and cycloheximide combined. Actinomycin D is an inhibitor of new RNA synthesis, and cycloheximide is an inhibitor of new protein synthesis. Therefore, the addition of actinomycin D ensured that the effects of O2 that we observed were due solely to the effects on mRNA stability and not to the continued effects of O2 on SP-A mRNA synthesis. Incubation of tissues in the presence of cycloheximide was designed to determine whether an observed effect of O2 on SP-A mRNA stability was modulated by the interaction of proteins with the SP-A mRNA transcript. SP-A mRNA half-lives were determined from these tissues through quantitation of autoradiograms generated from Northern blot analysis of SP-A mRNA. Data were normalized for 18S rRNA. As an additional control, the effect of O2 on the mRNA stability of constitutively expressed GAPD was also determined. The results are summarized in Table 1 and illustrated in Fig. 3. The SP-A mRNA half-life was increased significantly by 54% in tissues incubated in 70% O2 compared with the half-life in tissues incubated in 20% O2. The addition of cycloheximide to inhibit new protein synthesis had no effect on SP-A mRNA half-life in either 20% or 70% O2-incubated tissues. GAPD mRNA half-life was not different in tissues incubated in either 20% or 70% O2 or in those tissues incubated in either the absence or presence of cycloheximide. These data indicate a significant effect of O2 to increase SP-A mRNA half-life in human fetal lungs. Furthermore, the lack of an effect of cycloheximide is suggestive that new protein synthesis is not necessary for O2 to increase SP-A mRNA stability. Therefore, our data demonstrate that O2 regulates SP-A gene expression via both transcriptional and posttranscriptional mechanisms.

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<th>O2</th>
<th>SP-A mRNA t1/2, h</th>
<th>GAPD mRNA t1/2, h</th>
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<tr>
<td></td>
<td>Act D</td>
<td>Act D + CHX</td>
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<tr>
<td>20% O2</td>
<td>11.7 ± 1.9</td>
<td>10.5 ± 2.0</td>
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<tr>
<td>70% O2</td>
<td>18.0 ± 2.1*</td>
<td>21.5 ± 3.9*</td>
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Values are means ± SE of 4–6 independent half-life (t1/2) determinations by linear regression of all densitometric values over time corrected for loading and transfer errors by normalization to 18S rRNA levels. Tissues were incubated in presence of actinomycin D (Act D) without or with cycloheximide (CHX). SP-A, surfactant protein A; GAPD, glyceroldehyde-3-phosphate dehydrogenase. *P < 0.05 compared with 20% O2 condition.
Effect of O₂ on steady-state levels of SP-A1 and SP-A2 mRNA. To determine the effect of O₂ on the different genes for human SP-A (SP-A1 and SP-A2), we performed primer extension assays on mRNA from human fetal lung tissues incubated for 4 days in atmospheres of 20 or 70% O₂. We employed an oligonucleotide primer that hybridizes to nucleotides 1117–1144 in the SP-A1 gene (33) and nucleotides 1144–1171 in the SP-A2 gene (19). This primer is identical to primer B described by McCormick et al. (23). As such, the primer corresponds to exon I of SP-A1 and to exon II of SP-A2 (23). As shown in Fig. 4A, five bands representing primer extension products of ~161, 166, 201, 220, and 235 base pairs (bp) were reproducibly observed when mRNA from control (20% O₂ condition) human fetal lung explants was used as a template. Based on the size of the predicted mRNA splice variants, bands were identified as either SP-A1 or SP-A2 mRNA transcripts. The 161-bp band represents the major SP-A1 transcript, SP-A1α, which comprised 67 ± 3% of all SP-A1 mRNA transcripts in control explants (Fig. 4A). The 166-bp band represents a relatively minor SP-A1 transcript, SP-A1β, which comprised 26 ± 2% of all SP-A1 transcripts. Two minor SP-A1 transcripts, SP-A1γ and SP-A1δ, comigrated at 220 bp and constituted 5 ± 2% of the total SP-A1 mRNA pool. Two major SP-A2 mRNA transcripts, SP-A2α and SP-A2β, comigrated as 201-bp bands. These transcripts comprised 69 ± 5% of all SP-A2 transcripts observed in mRNA from control explants. Two minor SP-A2 transcripts, SP-A2γ and SP-A2δ, and one SP-A1 minor transcript, SP-A1ε, were represented by the 235-bp band. The SP-A2γ and SP-A2δ transcripts constituted 31 ± 5% of the SP-A2 mRNA pool, whereas SP-A1ε constituted 3% of the corresponding mRNA pool.

The effect of O₂ on the relative expression of total SP-A mRNA in the human fetal lung explants was evaluated in four experiments. Total SP-A mRNA was increased in the 70% O₂-treated tissues by 106 ± 62% compared with SP-A mRNA levels from tissues incubated in 20% O₂ (Fig. 4B). The effect of 70% O₂ on each of the various SP-A1 and SP-A2 mRNA transcripts was also evaluated (Fig. 4C). The mRNA levels for SP-A1α and SP-A1β were increased by 101 ± 51 and 138 ± 78%, respectively. The minor transcripts SP-A1γ and SP-A1ε were decreased by 10.2 ± 0.3%.
SP-A1δ together were increased by 128 ± 63%. The major SP-A2 transcripts SP-A2α and SP-A2β demonstrated a combined increase in expression of 118 ± 90%. The content of the three minor transcripts SP-A1ε, SP-A2γ, and SP-A2δ was also increased by 43 ± 85%. Thus an effect of O₂ to increase SP-A mRNA levels was observed in all of the various SP-A mRNA transcript subclasses. Furthermore, there was no differential effect of O₂ on any of the SP-A mRNA transcripts as the level of each transcript was increased approximately twofold in the 70% O₂ condition compared with the corresponding level of mRNA from the 20% O₂-treated tissues.

**DISCUSSION**

The present study demonstrates the ability of O₂ to increase mRNA levels for human SP-A by both transcriptional and posttranscriptional mechanisms. SP-A is one of a growing number of genes that are known to be induced by O₂. Other O₂-inducible genes that are expressed in the lung include those for the tissue inhibitor of metalloproteinases (16), manganese superoxide dismutase (MnSOD) (9), constitutive nitric oxide synthase (cNOS) (22), glutathione peroxidase (6), Na-K-adenosinetransphosphatase (ATPase)-α₁ and -β₁ (14), and the α₂, β₂, and γ-subunits of the epithelial Na channel (29). The mechanisms governing the induction of these genes by O₂ have been only partially explored. For example, MnSOD mRNA transcription is increased fourfold in the lungs of adult rats exposed to 85% O₂ for 3 days (15). Interestingly, MnSOD mRNA half-life is also increased over twofold in the lungs of rats exposed to hyperoxia (9). Glutathione peroxidase mRNA transcription rate increases over fivefold in human cardiac myocytes incubated in a Po₂ of 150 vs. 40 mmHg (10), although the half-life of glutathione mRNA in rat lung exposed to hyperoxia does not increase unless the animals are also injected with endotoxin (9). The effects of O₂ on Na-K-ATPase and epithelial Na channel gene transcription and/or mRNA stability have not been determined.

Transcription of human SP-A mRNA is increased by cAMP and glucocorticoid, with the greatest increase in transcriptional activity occurring in tissues incubated in the presence of both cAMP and glucocorticoid (4). The transcriptional response to cAMP by human SP-A genes is mediated by the binding of nuclear proteins, which are distinct from the cAMP response element binding protein, to specific DNA sequences within the 5'-flanking regions of the genes (34). Similarly, glucocorticoids may increase the transcription of human SP-A via binding of the glucocorticoid receptor to a consensus sequence, which has been identified in the 5'-flanking region of the gene (33). It has previously been determined that the O₂-induced increase in SP-A mRNA levels in the human fetal lung is not mediated by changes in the activity of cAMP-dependent protein kinase (1); however, several nuclear binding proteins that are known to activate gene transcription have been found to be induced by O₂ in the lung. These include c-Fos and c-Jun (activator protein-1), nuclear factor-κB, and CAAT-enhancer binding protein-β and -δ (7). Additionally, pulmonary lymphocytes from mice exposed to hyperoxia exhibit increased activation of nuclear factor-κB and increased levels of mRNA for interferon-γ (31). The promoter region of the human SP-A gene has binding sites for the CAAT-enhancer binding protein and interferon-γ (20), suggesting the possibility that the binding of one of these factors to the SP-A promoter may be responsible for the O₂-induced increase in SP-A gene transcription we observed in the present study.

The effect of O₂ to increase stability of SP-A mRNA by 54% in human fetal lung tissues incubated in 70% O₂ compared with tissues incubated in 20% O₂ further explains the ability of O₂ to increase steady-state levels of SP-A mRNA in this model of the developing human lung. Previously, the only factors known to regulate human SP-A mRNA stability were glucocorticoids. Incubation of human fetal lung explants in the presence of the synthetic glucocorticoid dexamethasone results in a significant decrease in SP-A mRNA half-life compared with control explants (5). This decrease in SP-A mRNA stability is reversed by coincubation of the tissues with the glucocorticoid-receptor antagonist RU-486, suggesting the possibility of a labile protein that stabilizes SP-A mRNA but is inhibited by binding of glucocorticoid to the glucocorticoid receptor (5). Further evidence for a labile protein factor that stabilizes SP-A mRNA is provided by Iannuzzi et al. (17), who, in similar studies with human fetal lung explants, found the dexamethasone-induced decrease in SP-A mRNA half-life to be reversed in tissues coincubated with cycloheximide, an inhibitor of new protein synthesis. We have found that the increase in human SP-A mRNA stability induced by O₂ is not modified by cycloheximide, suggesting that new protein synthesis is not necessary for stabilization of this mRNA by O₂. Therefore, the mechanism whereby the half-life of SP-A mRNA is increased by O₂ remains unclear but is not without precedent. For example, Lai et al. (22) found cNOS mRNA stability to be under negative control, as evidenced by an increased half-life of the cNOS mRNA in all O₂ tensions studied (3–95%), when bovine pulmonary arterial endothelial cells were incubated in the presence of cycloheximide. However, regardless of the absence or presence of cycloheximide, the mRNA half-life for cNOS increased with increasing O₂ concentrations in cells incubated in 3, 20, and 95% O₂. Taken together, this information suggests that O₂ may have a direct effect to stabilize the mRNA of some genes that is independent of the binding of protein factors to mRNA.

Although we have demonstrated the transcriptional and posttranscriptional regulation of SP-A mRNA levels by O₂ and we have shown that SP-A levels are also modulated by O₂, the physiological significance of our findings remains to be elucidated. Certainly, there is nothing “physiological” about the lung being exposed to any O₂ tension greater than that of ambient air, and one can argue that even 20% O₂ may constitute “hyperoxia” for the fetal lung. However, the existence of the regulatory mechanisms that we have observed in the
present study suggests that the regulation of SP-A levels by O2 may be of physiological importance. Previously, Acarregui and colleagues (1, 2) demonstrated that SP-A mRNA and SP-A levels are modulated throughout a range of O2 exposures (1–95%) in human fetal lungs maintained in vitro. The effect of O2 concentrations < 20% on the transcription rate and mRNA stability of human SP-A has not been evaluated; however, based on the earlier observations (1, 2) and the present investigation, we suspect that for SP-A mRNA stability of human SP-A has not been evaluated; how- ever, based on the earlier observations (1, 2) and the present investigation, we suspect that for SP-A mRNA either transcription rate, mRNA stability, or both are modulated throughout a range of O2 tensions. We speculate that the ability of O2 to increase SP-A levels may be important for adaptation of the newborn to extrauterine life or that SP-A levels may increase in vivo in response to increased oxygenation of tissues that may occur in association with vascularization of the developing lung.

Several different mRNA transcripts of both the SP-A1 and SP-A2 genes have been described (18, 23). The SP-A mRNA transcripts differ in the 5′ region of mRNA and arise as the result of differential splicing. A total of five SP-A1 and four SP-A2 transcripts have been described. Primer extension, with an oligonucleotide homologous to sequences present in all SP-A1 and SP-A2 mRNAs, can be used to identify the various SP-A1 and SP-A2 transcripts based on their length. We found that mRNAs for all transcripts of both human SP-A genes, SP-A1 and SP-A2, are increased in tissues incubated in 70% O2 compared with 20% O2-treated tissues. These data imply that our finding of O2 regulation of SP-A mRNA likely applies to both human SP-A genes. This regulation is in contrast to the effects of other SP-A gene regulators such as cAMP and glucocorticoids that differentially regulate the two human SP-A genes (24). Our data suggest that O2 regulates the steady-state levels of both SP-A1 and SP-A2 mRNA in a similar fashion, presumably by increasing both the transcription rate and mRNA stability of each gene product, although this assumption has not been proven. However, similar regulation of each gene by O2 implies that the elements required for regulation of these genes by O2 are shared by each of the human SP-A genes. Further investigation will be required to determine the elements required for O2 induction of SP-A gene expres- sion.

We acknowledge the technical assistance of Kieth L. Vogt and Kristie Ramirez. This research was funded by the American Lung Association; National Institute of Child Health and Human Development Grant HD-01116; National Heart, Lung, and Blood Institute Grant HL-50050; and National Institute of Diabetes and Digestive and Kidney Diseases Diabetes Endocrine Research Center Grant DK-25295.

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Received 23 Jul 1997; accepted in final form 2 December 1997.

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