Differential regulation of SP-A1 and SP-A2 genes by cAMP, glucocorticoids, and insulin

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Kumar, A. R., and J. M. Snyder. Differential regulation of SP-A1 and SP-A2 genes by cAMP, glucocorticoids, and insulin. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L177–L185, 1998.—In the human fetal lung, surfactant protein A (SP-A) is encoded by two highly similar genes, SP-A1 and SP-A2, which are developmentally and hormonally regulated. Using primer extension analysis, we evaluated the levels of SP-A1 and SP-A2 mRNA transcripts in human fetal lung explants and in a human adult lung adenocarcinoma cell line (H441 cells) cultured in the absence or presence of either dibutyryl adenosine 3',5'-cyclic monophosphate (DBcAMP, 1 mM), dexamethasone (10^{-7} M), or insulin (2.5 µg/ml). In the human fetal lung explants, the content of SP-A1 mRNA was approximately four times that of SP-A2 mRNA. DBcAMP increased SP-A1 mRNA levels by 100% and SP-A2 mRNA levels by 50%, thus reducing the ratio of SP-A1 mRNA to SP-A2 mRNA to ~1.1. Dexamethasone inhibited all of the SP-A1 and SP-A2 mRNA transcripts to the same extent, by ~70%, whereas insulin inhibited all SP-A mRNA transcripts by ~60%. The ratio of SP-A1 to SP-A2 mRNA in dexamethasone- or insulin-treated explants was the same as the ratio observed in controls. In the H441 cells, SP-A1 mRNA levels were ~1.5 times that of SP-A2 mRNA levels. DBcAMP increased both SP-A1 and SP-A2 mRNA levels by 100%. Dexamethasone inhibited SP-A1 mRNA levels in the cell line by 60%, whereas SP-A2 mRNA levels were not significantly affected. Insulin inhibited SP-A1 mRNA levels in the cell line by 40% without affecting SP-A2 mRNA levels. These findings suggest that the two human SP-A genes are regulated differently in the two model systems.

A critical event in the maturation of the fetus is the differentiation of lung alveolar type II cells (16). The alveolar type II cell synthesizes and secretes pulmonary surfactant, a lipoprotein substance that lines the alveolus and reduces surface tension at the air-fluid interface (32). Surfactant is composed of ~80% glycerophospholipids, 10% cholesterol, and 10% protein (32). Four surfactant-associated proteins (SPs) have been described, namely SP-A, SP-B, SP-C, and SP-D (30). These proteins have varied molecular characteristics and postulated functions, and at least the first three are required for proper surfactant function (30).

SP-A is the most abundant and best characterized SP (5). SP-A is synthesized as an ~35,000 dalton dial glycoprotein monomer that consists of an NH_{2}-terminal type IV collagen-like domain and a COOH-terminal lectin-like domain (6). The 35-kDa SP-A monomer forms trimers that subsequently aggregate in groups of six to form a flower bouquet-type structure (29). SP-A has sequence and structural homology with a group of proteins known as collectins, all of which are involved in host defense mechanisms (22). SP-A, together with SP-B and SP-C, has been shown to facilitate the surface tension-lowering properties of surfactant phospholipids (9). SP-A also acts as a homeostasis agent within the alveolus by regulating type II cell phospholipid synthesis, secretion, and recycling (15, 27, 33). More recently, evidence has accumulated that SP-A is involved in host defense mechanisms at the level of the alveolus against bacterial, viral, and fungal pathogens (28). Thus SP-A is an important surfactant component that contributes to many lung functions.

Two human SP-A genes and an SP-A pseudogene have been identified (13, 14, 31). Similarly, two SP-A genes have been identified in the baboon (7). In the human, the SP-A genes and pseudogene are located in a locus on chromosome 10, q21-q24 (1). The two human SP-A genes are 94% identical in their nucleotide sequence and encode proteins that differ by eight amino acids (13). It has been suggested that the SP-A protein trimers that make up the flower bouquet structure are heterotrimers that consist of two SP-A1 molecules and one SP-A2 molecule (29). Five SP-A1 mRNA transcripts and four SP-A2 mRNA transcripts have been described (12, 17). The various SP-A1 and SP-A2 mRNA transcripts differ in the 5'-untranslated region of the mRNA (12, 17). The functional significance of these different SP-A mRNA transcripts is currently not understood.

SP-A is induced in the fetal lung type II cell toward the end of gestation and is regulated in a biphasic manner by glucocorticoids, hormones that are used clinically to accelerate lung development (20, 24). At relatively high concentrations (\geq 10^{-7} M), dexamethasone decreases SP-A mRNA and protein levels in human fetal lung explants (20). In contrast, it has been shown that high concentrations of insulin inhibit SP-A gene expression (3). The studies described above were performed using methods that detected both SP-A gene products together. In a recent study, McCormick and Mendelson (18) described the effects of dibutyryl adenosine 3',5'-cyclic monophosphate (DBcAMP) alone or with dexamethasone, a synthetic glucocorticoid, on SP-A1 and SP-A2 gene expression in human fetal lung explants (18).

In the fetal baboon lung, SP-A1 mRNA is detected before SP-A2 mRNA (7). In addition, in lung tissue obtained from a 28-wk human neonate who died shortly after birth, SP-A1 mRNA, but not SP-A2 mRNA, was detectable by primer extension analysis (18). It was...
shown that DBcAMP increased levels of SP-A2 mRNA to a greater extent than SP-A1 mRNA (18). Dexamethasone at 10^{-7} M inhibited the stimulatory effect of DBcAMP on SP-A2 mRNA but had less of an effect on SP-A1 mRNA (18). The effects of glucocorticoids alone on either SP-A1 mRNA or SP-A2 mRNA levels have not yet been described, nor has the effect of insulin.

In the present study, we examined the effects of DBcAMP, dexamethasone, and insulin on the relative amounts of SP-A1 and SP-A2 mRNA transcripts. We used two model systems to conduct the study, i.e., human fetal lung explants (25) and the H441 cell line (8). H441 cells are a pulmonary adenocarcinoma cell line that expresses both SP-A mRNA and SP-B mRNA (8). We found that the three physiological mediators, glucocorticoids, cAMP, and insulin, regulated both human SP-A genes. In addition, the various mRNA transcripts within each SP-A gene class were also regulated by these mediators. Finally, we found that, in some respects, the regulation of the two SP-A genes differed in the human fetal lung explants compared with the H441 cell line.

**MATERIALS AND METHODS**

Human organ culture. The experimental protocol for this study was approved by the Human Research Review Committee at the University of Iowa. Human fetal lung explants were cultured in vitro essentially as previously described (25). Explants were cultured in Waymouth's MB 752/1 media without additives (control) or in media containing one of the following substances: DBcAMP (1 mM), dexamethasone (10^{-7} M), or human insulin (2.5 μg/ml). Stock solutions were prepared as follows: DBcAMP (Sigma Chemical, St. Louis, MO) was dissolved in sterile water at a concentration of 100 mM and stored at −20°C; dexamethasone (Sigma) was dissolved in ethanol at a concentration of 10^{-3} M and stored at −20°C; insulin (Boehringer Mannheim, Indianapolis, IN) was solubilized in 0.1 N HCl at a concentration of 2 mg/ml and stored at −70°C. Media were changed daily. Explants were harvested after 6 days in culture, frozen rapidly in liquid nitrogen, and stored at −70°C until used for analysis.

Cell culture. H441 cells derived from a human lung adenocarcinoma cell line were maintained in RPMI 1640 medium (GIBCO BRL Life Technologies, Grand Island, NY) containing 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B, and 10% fetal bovine serum in 100-mm tissue culture dishes (19). Cells were allowed to grow to confluency (usually 7 days) and then passed after a 1:4 split. Media were changed 4 days after subculturing. Experiments were carried out on confluent cells (day 7 of culture). One day before the experiment, cells were transferred to serum-free RPMI 1640 media. This 24-h preincubation was followed by the replacement of the media with either serum-free media containing no additives (control) or media containing DBcAMP (1 mM), dexamethasone (10^{-7} M), or human insulin (2.5 μg/ml). Media containing the regulatory factors were removed after 24 h of incubation, and the cells were removed from the dishes using a rubber scraper, pelleted by centrifugation, frozen, and stored at −70°C until analysis.

RNA isolation and Northern blot analysis. Total RNA was isolated from the cultured fetal lung explant tissue obtained from one 35-mm tissue culture dish and from one 100-mm tissue culture dish of H441 cells using the single-step method of Chomczynski and Sacchi (2). RNA was quantitated by determining the absorbance at 260 nm, and purity was assessed by determining the ratio of absorbance at 260 nm to that at 280 nm. Ten micrograms of total RNA from each sample were separated by gel electrophoresis on a 1.2% agarose-5% formaldehyde gel. A Polaroid photograph of the ethidium bromide-stained gel was obtained under ultraviolet (UV) light. The RNA was transferred by capillary action to a positively charged nylon membrane (Nytran plus, Schleicher and Schuell, Keene, NH) and cross-linked to the membrane by UV irradiation (GS Gene Linker UV Chamber; Bio-Rad Life Sciences, Hercules, CA). A cDNA probe for human SP-A was radiolabeled with α-32P]deoxyxytidine triphosphate (3,000 Ci/mmol; Amersham Life Science, Bedford, MA) using a random-primer kit (Boehringer Mannheim). The 0.9-kb SP-A cDNA probe was obtained from Dr. J effrey Whitsett (University of Cincinnati, Cincinnati, OH). Northern blot analysis was performed essentially as described previously (3). Reactive bands on the X-ray film were quantitated using scanning densitometry. Loading errors were corrected by densitometric quantitation of the 18S ribosomal RNA bands on the photograph of the ethidium bromide-stained agarose gel.

Northern blot analysis using oligonucleotide probes. Northern blot analysis using oligonucleotide probes was performed using protocols described by Henderson et al. (10). RNA (20 μg) from control and DBcAMP-treated human fetal lung explants was analyzed for SP-A1 and SP-2 mRNA. Oligonucleotide probes specific for SP-A1 and SP-A2, as recently described (11), were synthesized. The oligonucleotides were labeled by the addition of a homopolymeric tail of [α-32P] deoxyadenosine residues to their 3'-end using terminal deoxynucleotidyltransferase (Promega, Madison, WI) and [α-32P]dATP (6,000 Ci/mmol; Amersham). Unincorporated nucleotides were removed with a Sephadex G-25 spin column (Boehringer Mannheim). The membranes were prehydrated, hybridized, and washed essentially as described by Henderson et al. (10). The membranes were then air-dried and exposed to Kodak X-AR film between two intensifying screens (DuPont) at −70°C.

Primer extension analysis. An oligonucleotide primer (GGG-GATAACGGGCTTCAAACGAACG) that is complementary to nucleotides 1117–1144 of the human SP-A1 gene (31) and nucleotides 1144–1171 of the human SP-A2 gene (13) was labeled at its 5’-end using [γ-32P]ATP (5,000 Ci/mmol; Amersham) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA), as described (17, 23). The unincorporated radioactive nucleotide was removed with a Sephadex G-25 spin column (Boehringer Mannheim). Primer extension was carried out using a modification of a standard protocol (23). Five micrograms of total RNA were incubated with 0.2 pmol of the radioactively labeled primer for 10 min at 70°C and then cooled on ice for 10 min. The primer was extended using 200 units of Superscript II reverse transcriptase (GIBCO BRL) at 42°C for 1 h in 20 µl of buffer containing 50 mM tris(hydroxymethyl) aminomethane hydrochloride (pH 8.3), 75 mM KCl, 3 mM MgCl2, 1 mM each dATP, dGTP, dCTP, and dTTP, 20 units of ribonuclease inhibitor, 1 mM dithiothreitol, and 50 µg/ml actinomycin D. The extended transcripts were recovered as described (23) and separated on a 6% polyacrylamide-7 M urea sequencing gel using standard procedures (23). The gel was dried on a gel drier (Bio-Rad Life Sciences) and exposed to Kodak X-AR film with an intensifier screen (Lighting Plus, DuPont, Wilmington, DE) at −70°C. Band densities were quantitated by densitometric analysis.

Data analysis. Experiments were repeated three to seven times. Results are based on band densities, measured as contour (mean optical density × area) using a scanner and...
RESULTS

As illustrated in Fig. 1, the amount of SP-A mRNA detected in primer extension assays from six different cultured fetal lung samples varied considerably from experiment to experiment. Although not all of the bands were visible in every lane depicted in Fig. 1, in the individual experiments, exposures were adjusted so that all bands were visible. Two nomenclatures have been proposed for the naming of the various SP-A mRNA transcripts (Table 1; see Refs. 12, 17). The 235-bp band represents three relatively minor splice variants, one SP-A1 (SP-A1a) and two SP-A2 (SP-A2a, SP-A2b), that comigrate and were not resolved on the sequencing gel used in this study. Two closely migrating minor SP-A1 transcripts (SP-A1γ, δ) migrated at 220 bp. A major SP-A2 transcript of ~201 bp was identified, which may represent two very similar, closely migrating SP-A2 transcripts (SP-A2α, β; see Refs. 12 and 17). A major SP-A1 transcript of 161 bp (SP-A1α) and a minor SP-A1 transcript of 166 bp (SP-A1β) were also identified in all control RNA samples. In control explants, SP-A1α comprised the majority of all SP-A1 transcripts, whereas the comigrating SP-A2α, β transcripts comprised the majority of all the SP-A2 mRNA transcripts (Table 1). In seven experiments, each performed using cultured human fetal lung explant tissue obtained from a different fetus, we found that SP-A1α constituted ~75% of the major SP-A mRNA transcripts present (Table 2). The ratio of SP-A1α mRNA levels to SP-A2α, β mRNA levels in the individual experiments ranged from 2.06 to 7.75, with a mean ratio of 4.9 ± 0.7.

Human fetal lung experiments. The effects of three physiological mediators, DBcAMP, dexamethasone, and insulin, on the relative expression of the SP-A genes in human fetal lung explants were evaluated by primer extension analysis in five experiments (Fig. 2). DBcAMP significantly increased total SP-A mRNA by 195.8 ± 96.3%, whereas dexamethasone significantly decreased total SP-A mRNA by 78.4 ± 9.9%, and insulin also significantly decreased total SP-A mRNA by 67.4 ± 0.8% (P < 0.05, Dunnett’s test).

The effects of three physiological mediators on the relative amounts of the major SP-A1 and SP-A2 mRNA transcripts in the total explant SP-A mRNA pool were also evaluated. As shown in Fig. 3A, DBcAMP significantly increased the levels of SP-A2α, β mRNAs (569.6 ± 273.5%) when compared with controls (P < 0.05, Dunnett’s test). The response of the SP-A2 gene in human fetal lung explants to DBcAMP was variable and ranged from an increase of 200 to 1,200%. The effect of DBcAMP on SP-A1 mRNA levels was not significant. Dexamethasone significantly decreased the relative amount of the SP-A1α and SP-A2α, β mRNA transcripts (by 78.8 ± 9.8 and 82.2 ± 9.3%, respectively; Fig. 3A). Insulin also significantly decreased the SP-A1α and SP-A2α, β mRNA transcript levels (59.8 ± 7.7 and 67.2 ± 13.2%, respectively) when compared with controls (Fig. 3A). The inhibitory effects of insulin and dexamethasone on either SP-A1α mRNA or SP-A2α, β mRNA did not differ in magnitude.

DBcAMP significantly decreased the proportion of the major SP-A1 mRNA transcript (SP-A1α) and significantly increased the proportion of the major SP-A2 mRNA transcripts (SP-A2α, β) in the total pool of surfactant protein (SP) A mRNA present in control human fetal lung explants. Lanes 1–6 represent primer extension results from 6 different experiments in which 5 µg of RNA from each sample were used. Arrows point to the bands representing the indicated SP-A1 or SP-A2 mRNA transcripts. The relative amount of SP-A mRNA present in the 6 different control human fetal lung explants varied considerably.

Fig. 1. Autoradiograph of primer extension analysis of surfactant protein (SP) A mRNA present in control human fetal lung explants. Lanes 1–6 represent primer extension results from 6 different experiments in which 5 µg of RNA from each sample were used. Arrows point to the bands representing the indicated SP-A1 or SP-A2 mRNA transcripts. The relative amount of SP-A mRNA present in the 6 different control human fetal lung explants varied considerably.
explain SP-A mRNA (Table 2). Dexamethasone, at 10^{-7} M, which is an inhibitory concentration, did not significantly alter the percentage of the major SP-A1 and SP-A2 mRNA transcripts present in the explants when compared with controls (Table 2). Insulin also had no significant effect on the percentage of SP-A1 and SP-A2 major mRNA transcripts in the explants when compared with controls (Table 2).

We evaluated the effects of DBcAMP, dexamethasone, and insulin on the three minor SP-A transcript bands observed in the primer extension assay (Fig. 2). In mRNA from control explants, the SP-A1 \(\beta\) transcript comprised \(\sim 25\%\) of the total SP-A1 mRNA detected, the SP-A1 \(\gamma,\delta\) transcripts comprised \(\sim 5\%\) of the total SP-A1 mRNA, and the 235-bp band, which consists of two SP-A2 (SP-A2 \(\gamma,\delta\)) and one SP-A1 transcript (SP-A1 \(\epsilon\)), constituted \(\sim 2.5\%\) of the total SP-A1 mRNA and \(25.9 \pm 0.01\%\) of the SP-A2 mRNA transcripts (Table 1). As shown in Fig. 3B, the SP-A1 \(\beta\), SP-A1 \(\gamma,\delta\), SP-A2 \(\gamma,\delta\) transcripts were all increased by DBcAMP, although the effects were not significant. In contrast, the relative amounts of all of the minor SP-A1 and SP-A2 mRNA transcripts were decreased significantly by dexamethasone and insulin (Fig. 3B).

H441 cell line experiments. Essentially the same bands representing the nine different SP-A1 and SP-A2 mRNA transcripts were detected in primer extensions of RNA isolated from the cell line (Fig. 4). As shown in Table 1, the SP-A1 \(\alpha\) mRNA transcript comprised the

### Table 1. Proportion of SP-A mRNA transcripts in human fetal lung explants and in H441 cells

<table>
<thead>
<tr>
<th>Gene Splice Variant</th>
<th>Explants (n=7)</th>
<th>Cell Line (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha) (AD')</td>
<td>68.1 ± 1.8</td>
<td>63.8 ± 2.1</td>
</tr>
<tr>
<td>(\beta) (AD')</td>
<td>24.9 ± 1.0</td>
<td>32.0 ± 2.3*</td>
</tr>
<tr>
<td>(\gamma,\delta)</td>
<td>4.6 ± 1.7</td>
<td>1.8 ± 1.8*</td>
</tr>
<tr>
<td>(\epsilon) (A'B'D')</td>
<td>2.4 ± 0.3</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>SP-A2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha + \beta)</td>
<td>74.1 ± 0.1</td>
<td>81.7 ± 8.3</td>
</tr>
<tr>
<td>(\gamma,\delta)</td>
<td>25.9 ± 0.1</td>
<td>18.3 ± 8.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of experiments. Units are %. SP-A, surfactant protein A. Greek letters indicate nomenclature as described by McCormick et al. (17). Capital letters in parentheses indicate nomenclature as described by Karinch and Flores (12). *Significantly different from human fetal lung explant data, \(P < 0.05\), Student’s t-test.

Fig. 2. Primer extension analysis of SP-A mRNA transcripts in 5 µg of total RNA isolated from human fetal lung explants treated for 6 days with either no additions (C), dibutyryladenosine 3',5'-cyclic monophosphate [DBcAMP (DB), 1 mM], dexamethasone (DEX, 10^{-7} M), or insulin (I, 2.5 µg/ml). Bands representing the different SP-A1 and SP-A2 mRNA transcripts are labeled on the left. The results are from a representative experiment.

Fig. 3. Effects of regulatory factors (DBcAMP, dexamethasone, and insulin) on relative amounts of SP-A1 and SP-A2 mRNA transcripts in human fetal lung explants. A: relative amount of the major SP-A1 mRNA transcript, SP-A1 \(\alpha\), and the major SP-A2 mRNA transcripts, SP-A2 \(\alpha,\beta\), in explants treated with either no additions (control), DBcAMP, dexamethasone, or insulin for 6 days. B: relative amount of SP-A1 \(\beta\) mRNA, SP-A1 \(\gamma,\delta\), SP-A1 \(\epsilon\), and SP-A2 \(\gamma,\delta\) mRNA transcripts in explants treated with either no additions (control), DBcAMP, dexamethasone, or insulin for 6 days. Data were obtained by densitometric analysis of 5 different experiments. Values were normalized to the control condition in each experiment, which was made equal to one. Data represent means ± SE of 5 experiments. *Significant difference from control condition (\(P < 0.05\), Dunnett’s test).
majority of the total SP-A1 mRNA pool. The SP-A2 α and β mRNA transcripts together comprised the majority of the total SP-A2 mRNA transcripts (Table 1). The proportions of most of the SP-A1 and SP-A2 mRNA transcripts observed in the H441 cell line did not differ significantly from that observed in the human fetal lung explants (Table 1). The exceptions were the SP-A1 β transcript, which represented a greater proportion of the SP-A1 mRNA in the H441 cell line than in the explants, and the SP-A1γ,δ transcripts, which were less abundant in the H441 cell line than in the human fetal lung explants (Table 1).

The effects of DBcAMP, dexamethasone, and insulin on the levels of total SP-A mRNA as detected by primer extension in the H441 cell line were evaluated in five experiments. DBcAMP significantly increased total SP-A mRNA by 97.8 ± 29.0%, whereas dexamethasone significantly decreased the total amount of SP-A mRNA detected in the H441 cells by 62.0 ± 10.2%. Insulin also significantly decreased the total SP-A mRNA pool by 55.5 ± 9.7% in the H441 cell line.

The relative amounts of the SP-A1α and SP-A2α,β mRNA transcripts in the H441 cell line were significantly increased by DBcAMP (72.2 ± 20.6 and 92.6 ± 27.7%, respectively; Fig. 5A). Dexamethasone significantly decreased the relative amount of SP-A1α mRNA by 63.2 ± 10.4%, and insulin significantly decreased SP-A1α mRNA by 38.4 ± 13.2% in the H441 cells (Fig. 5A). Dexamethasone and insulin had no significant effect on SP-A2α,β mRNA levels in the H441 cell line (Fig. 5A).

Table 2. Relative proportions of the major SP-A1 and SP-A2 mRNA transcripts in the total SP-A mRNA pool in human fetal lung explants and in H441 cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>SP-A1α</th>
<th>SP-A2α + β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human fetal lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76.2 ± 4.4</td>
<td>23.8 ± 4.4</td>
</tr>
<tr>
<td>DBcAMP</td>
<td>58.9 ± 5.3*</td>
<td>41.1 ± 5.3*</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>83.8 ± 3.2</td>
<td>16.2 ± 3.2</td>
</tr>
<tr>
<td>Insulin</td>
<td>78.1 ± 6.3</td>
<td>21.9 ± 6.3</td>
</tr>
<tr>
<td>H441 cell line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>58.1 ± 2.1</td>
<td>41.7 ± 2.1</td>
</tr>
<tr>
<td>DBcAMP</td>
<td>52.8 ± 3.0</td>
<td>47.2 ± 3.0</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>37.0 ± 5.7*</td>
<td>63.0 ± 5.7*</td>
</tr>
<tr>
<td>Insulin</td>
<td>46.2 ± 3.4*</td>
<td>53.8 ± 3.4*</td>
</tr>
</tbody>
</table>

Data are means ± SE in %. DBcAMP, dibutyryladenosine 3',5'-cyclic monophosphate. *Significantly different from control, P < 0.05, Dunnett’s test.

Fig. 4. Primer extension analysis of SP-A mRNA transcripts in 5 µg of total RNA isolated from H441 cells treated for 24 h with either no additions (C), 1 mM DBcAMP (DB), 10−7 M dexamethasone, or 2.5 µg/ml insulin. Bands representing the different SP-A1 and SP-A2 mRNA transcripts are labeled on the left. Data are from a representative experiment.

Fig. 5. Effects of regulatory factors (DBcAMP, dexamethasone, and insulin) on relative amounts of SP-A1 and SP-A2 mRNA transcripts in H441 cells. A: relative amount of the major SP-A1 mRNA transcript, SP-A1α, and the major SP-A2 mRNA transcripts, SP-A2α,β, in H441 cells treated with either no additions (control), DBcAMP, dexamethasone, or insulin for 24 h. Data were obtained by densitometric analysis of 5 different experiments. Values were normalized to the control condition in each experiment, which was made equal to one. Data represent means ± SE of 5 experiments. *Significant difference from corresponding control condition (P < 0.05, Dunnett’s test).
SP-A1α mRNA comprised ~60% of the major SP-A mRNA transcripts (Table 2). The proportions of the major SP-A1 and SP-A2 mRNA transcripts were not significantly affected by DBcAMP in H441 cells (Table 2). In contrast, dexamethasone and insulin significantly decreased the proportion of SP-A1α mRNA and significantly increased the proportion of SP-A2α,β mRNA transcripts present in the cell line (Table 2).

SP-A1β was significantly increased 112.2 ± 34.3% by DBcAMP in the H441 cell line (Fig. 5B). Dexamethasone treatment significantly decreased the minor SP-A1β transcript by 69.0 ± 8.5%, whereas insulin had no significant effect (Fig. 5B). SP-A1γ and -δ mRNA transcripts were only detected in two of the five cell line experiments. Thus the effects of the mediators on these transcripts could not be determined. DBcAMP significantly increased the proportion of the major SP-A1α mRNA comprised ~60% of the total SP-A1 mRNA, a value similar to that reported previously by McCormick et al. (17) and slightly lower than the 81% reported by Karinch and Floros (12). For SP-A2 mRNA, the major transcripts, SP-A2α and SP-A2β, which were not resolved in our primer extension analysis, comprised 78% of the total SP-A2 mRNA. In contrast, both previous reports estimated that these transcripts were 93% of the total SP-A2 mRNA in human lung (12, 17).

McCormick et al. (17) reported the ratio of the major SP-A1 transcripts to SP-A2 transcripts was ~1.9 in human fetal lung explants, with no range reported, and a ratio of ~0.33 in adult human lung (n = 4). Karinch et al. (11) reported a mean ratio of 4.7 in adult human lung tissue, with a range of 0.9–6.8 (n = 21). We observed a ratio of ~4.9 ± 0.72, with a range of ~2.1–7.8 (n = 7), in control human fetal lung explants. The observed variation in ratios of SP-A1 to SP-A2 mRNA may reflect variability in the human population. Karinch et al. (11) have postulated that the ratio of the SP-A1 to SP-A2 major transcripts is a parameter that is influenced by the SP-A1 and SP-A2 alleles expressed by each individual. In the H441 cells, we calculated a SP-A1-to-SP-A2 mRNA ratio of 1.6 ± 0.2 (n = 5 experiments); the range was 1.2–2.3. The narrow range of the ratios of SP-A1 to SP-A2 mRNA observed in the H441 cells probably reflects the clonal nature of the cells, which are expressing one genotype.

Validation of primer extension analysis. To validate the use of primer extension analysis to measure the relative abundance of SP-A1 vs. SP-A2 mRNA, we performed Northern blot analysis using oligonucleotide probes specific for SP-A1 mRNA or SP-A2 mRNA (Fig. 6A). As shown in Fig. 6A, SP-A1 mRNA was more easily detected in control human fetal lung explants than SP-A2 mRNA. Densitometric analysis of the oligonucleotide blot showed that SP-A1 mRNA comprised ~60% of the SP-A mRNA detected in controls (Fig. 6C, left). Similar results were obtained when the relative amounts of SP-A1 and SP-A2 mRNAs present in the control explants were determined by primer extension analysis (Fig. 6, B and C, right). We observed that DBcAMP increased the levels of SP-A1 mRNA approximately twofold and the levels of SP-A2 mRNA about sevenfold in the oligonucleotide Northern blot analysis (Fig. 6C, left). In the primer extension analysis of the same sample, DBcAMP increased SP-A1 mRNA about twofold and increased SP-A2 mRNA about fivefold (Fig. 6C, right). Thirty different RNA samples obtained from five explant experiments and five cell line experiments were analyzed by both primer extension analysis and Northern blot analysis for total SP-A mRNA. The relative amount of total SP-A mRNA as determined by primer extension analysis was plotted against the relative amount of total SP-A mRNA as determined by Northern blot analysis (Fig. 6D). The total SP-A mRNA quantitations, as determined by the two different methods, were highly correlated (r = 0.91, P < 0.05).

DISCUSSION

Estimates of the proportions of the various SP-A transcripts in total lung mRNA have been made previously using rapid amplification of cDNA ends (12, 17). To a great extent, the proportions determined in the present study, using densitometry, agree with the previously reported data (12, 17). For SP-A1 mRNA, we determined that the major transcript, SP-A1α, was ~65% of the total SP-A1 mRNA, a value similar to that
secondary effects on epithelial cells mediated by the effects of dexamethasone on nonepithelial cells present in the human fetal lung explants. Alternatively, because the H441 cell line is derived from a lung tumor, it may differ from normal lung epithelial cells with respect to its response to glucocorticoids.

In the human fetal lung explants, there was no significant effect of insulin on the relative proportion of SP-A1 vs. SP-A2 mRNA. However, in the H441 cell line, insulin significantly decreased the proportion of SP-A1 mRNA and significantly increased the proportion of SP-A2 mRNA. It is interesting to note that both inhibi-
ulatory regulators of SP-A gene expression, i.e., dexamethasone and insulin, had no significant effect on the relative proportions of SP-A1 vs. SP-A2 mRNA in the human fetal lung explant system. However, in the H441 cells, both inhibitors decreased the proportion of SP-A1 mRNA and increased the proportion of SP-A2 mRNA.

In the present study, we also evaluated the effects of the physiological mediators on the minor SP-A transcripts, SP-A1β, SP-A1γ, SP-A1ε, and SP-A2γ,δ. In the human fetal lung explants, DBcAMP did not significantly increase levels of any of the minor SP-A transcripts. In contrast, in the H441 cell line, SP-A1ε and the SP-A1γ,δ mRNA transcripts were significantly increased by DBcAMP. In the explants, dexamethasone and insulin inhibited the levels of all of the minor SP-A transcripts. In contrast, in the H441 cells, the only significant effect observed was that of dexamethasone on SP-A1β. The SP-A protein encoded by the SP-A1γ and -ε and SP-A2γ,δ transcripts may have a slightly different amino acid sequence and thus may serve a different function (17).

cAMP tended to increase the expression of both human SP-A genes, whereas both were inhibited by dexamethasone (10⁻⁷ M) and insulin. However, the SP-A gene was more sensitive to DBcAMP treatment than the SP-A1 gene in the human fetal lung explants but not in the H441 cell line. The ratio of SP-A1 mRNA to SP-A2 mRNA was ∼5:1 in control explants and ∼1:1 in DBcAMP-treated explants. In the H441 cell line, the ratio in control cells is ∼1:1, and it is not changed by DBcAMP treatment. Whether a change in the relative abundance of the two different SP-A mRNAs is reflected in the proportions of SP-A1 and SP-A2 proteins remains to be determined. It has been proposed that the native SP-A protein exists as an octadecamer composed of six SP-A heterotrimer, each of which consists of two SP-A1 molecules and one SP-A2 molecule (29). Although alternative molecular structures of SP-A have not been reported, our data are consistent with the possibility that different SP-A protein trimers or other aggregates may exist. In the DBcAMP-treated explants, in which the ratio of SP-A1 mRNA to SP-A2 mRNA is 1:1, one might speculate that the two SP-A proteins might also exist in a 1:1 ratio and that structures other than the putative SP-A heterotrimer might be formed. The existence and putative function of such postulated SP-A molecules remains to be investigated. A dimeric form of SP-A, which is not reduced during sodium dodecyl sulfate-polyacrylamide gel electrophoresis, has been consistently observed in alveolar proteinosis samples (26). In contrast to the disparate effects of DBcAMP on the two human SP-A genes observed in the human fetal lung explants, dexamethasone and insulin inhibited both SP-A genes in the human fetal lung explants in a remarkably similar manner.

We used the H441 cell line in this study because it is an epithelial cell line and as such is a simpler system than the human fetal lung explants, which consist of many different cell types. The H441 cell line expresses SP-A and SP-B mRNA and protein, and the regulation of the SP genes in the H441 cell line appears to be very similar to that previously reported using the human fetal lung explant system (19). However, we did find some differences in the regulation of the SP-A2 gene by dexamethasone and insulin in the two model systems. Dexamethasone and insulin inhibited the SP-A2 gene much more profoundly in the human fetal lung explants than in the H441 cells.

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