Regulation of Expression of the Gene Encoding the Major Surfactant Protein (SP-A) in Human Fetal Lung in Vitro

DISPARATE EFFECTS OF GLUCOCORTICOIDS ON TRANSCRIPTION AND ON mRNA STABILITY*

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We previously observed that dexamethasone had a biphasic effect on the levels of mRNA encoding the major surfactant protein (SP-A) in human fetal lung in vitro; at concentrations of \(10^{-10}\) and \(10^{-9}\) M, dexamethasone caused an increase in the levels of SP-A mRNA, whereas at concentrations of \(>10^{-8}\) M, the steroid had a pronounced inhibitory effect on SP-A mRNA levels. It was also found that dexamethasone antagonized the stimulatory effect of dibutyryl cyclic AMP (Bt2cAMP) on SP-A mRNA levels in human fetal lung in vitro. It was our objective, in the present study, to characterize further the effects of dexamethasone and Bt2cAMP on SP-A mRNA levels in human fetal lung tissue and to determine whether such effects are associated with comparable changes in the transcriptional activity of the SP-A gene. We found that the action of dexamethasone \((10^{-7}\) M\) to reduce the levels of SP-A mRNA in control and Bt2cAMP-treated fetal lung explants was evident within 2 h of its addition to the culture medium; SP-A mRNA was reduced to barely detectable levels in control and in Bt2cAMP-treated tissues after 24 h of dexamethasone treatment. The action of dexamethasone to reduce SP-A mRNA levels was not prevented by co-incubation with either actinomycin D or cycloheximide. In contrast to its dose-related biphasic effects on the levels of SP-A mRNA, we found that dexamethasone caused a dose-dependent stimulation of SP-A gene transcription. Bt2cAMP also increased the transcriptional activity of the SP-A gene in the human fetal lung in vitro. In fetal lung explants incubated in the presence of dexamethasone plus Bt2cAMP, a synergistic induction of SP-A gene transcription was observed at concentrations of dexamethasone of \(10^{-9}-10^{-7}\) M. Our findings are indicative that the stimulatory effects of dexamethasone \((10^{-10}-10^{-9}\) M\) on SP-A mRNA levels are reflective of a stimulatory effect of the steroid on SP-A gene transcription, whereas the inhibitory effects of dexamethasone \((10^{-7}\) M\) on SP-A mRNA levels are the result of a dominant effect of the steroid in elevated concentrations to reduce SP-A mRNA stability.

The synthesis of pulmonary surfactant, a lipoprotein comprised of 80% glycerophospholipid and 5–10% protein, is developmentally regulated in fetal lung tissue. Surfactant synthesis by the fetal lung, which is initiated during the latter 10–15% of gestation in all mammalian species, is believed to be hormonally regulated. A number of hormones, including glucocorticoids, prolactin, thyroid hormones, and estrogens, have been reported to enhance the synthesis of surfactant glycerophospholipids in fetal lung tissue (see Ref. 1 for review).

The surfactant-associated proteins also are subject to multifactorial regulation. The major surfactant-associated protein, SP-A, a 35,000-dalton glycoprotein \((2-4)\), binds strongly to surfactant glycerophospholipids promoting their aggregation \((5)\) and may serve a role together with calcium in the structural organization of tubular myelin \((6,7)\), a lattice-like structure that appears to facilitate the rapid formation of the phospholipid surface film at the alveolar air-liquid interface. SP-A may also bind to specific receptors on the luminal surface of the type II cells and mediate the reutilization of secreted surfactant glycerophospholipids and proteins \((8)\). Cyclic AMP analogues markedly stimulate the synthesis of SP-A and increase the levels of its mRNA in both rabbit \((9,10)\) and human \((11,12)\) fetal lung tissue maintained in organ culture. In fetal rabbit lung in culture, the stimulatory effects of Bt2cAMP\(^1\) on SP-A mRNA levels are associated with comparable increases in the transcriptional activity of the SP-A gene \((13)\). The stimulatory effects of Bt2cAMP on SP-A synthesis in human fetal lung in vitro are mimicked by the \(\beta\)-adrenergic agonist, terbutaline \((11)\). We also have observed that insulin causes a dose-dependent inhibition of the accumulation of immunoreactive SP-A in human fetal lung in vitro \((14)\).

Glucocorticoids also have been reported to regulate the synthesis of SP-A in fetal lung tissue in vitro and in vivo; however, the reported effects of glucocorticoids on SP-A synthesis and on the levels of its mRNA are conflicting. We observed that cortisol treatment of lung explants from 21-day gestational age fetal rabbits caused an increase in the levels of SP-A and its mRNA \((9)\). Phelps et al. \((15)\) recently reported that dexamethasone administration to fetal and neonatal rats increased SP-A synthesis and the levels of its mRNA. In studies with human fetal lung in vitro, Ballard and colleagues \((16)\) reported that dexamethasone at \(10^{-8}\) M caused a marked stimulation of the levels of SP-A and its mRNA. In contrast, Whitsett et al. \((12)\) reported that dexamethasone caused a dose-dependent inhibition of accumulation of SP-A and SP-A mRNA. On the other hand, we have found that dexamethasone has a biphasic effect on the levels of SP-A and its mRNA in human fetal lung in culture \((17)\); at concentrations of \(10^{-10}\) and \(10^{-9}\) M, dexamethasone has a stimulatory effect, whereas at concentrations greater than \(10^{-8}\) M, the glucocor-

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1 The abbreviation used is: Bt2cAMP, dibutyryl cyclic AMP.
ticoid is markedly inhibitory as compared with control tissues. These biphase effects of dexamethasone are also observed in the presence of Bt2cAMP (17). Liley et al. (18) also recently reported that dexamethasone has dose-dependent biphase effects on the levels of SP-A and its mRNA in human fetal lung in vitro; however, in those studies the levels of SP-A protein and mRNA in lung explants treated with dexamethasone at $10^{-7}$ M were comparable with those of control tissues. These investigators also reported that the stimulatory effects of dexamethasone ($10^{-8}$ M) on SP-A accumulation were manifest after relatively brief (24-72 h) periods of incubation; after longer incubation times, these stimulatory effects were no longer apparent (18).

In the present investigation, we have assessed the effects of dexamethasone on the transcriptional activity of the SP-A gene and on the associated levels of SP-A mRNA in human fetal lung in vitro to define the molecular mechanisms whereby glucocorticoids elicit their effects on the levels of SP-A mRNA in this tissue. We have observed that dexamethasone has a paradoxical effect on SP-A gene expression in human fetal lung. The glucocorticoid causes a rapid dose-dependent decrease in SP-A gene transcription; however, at concentrations of $>10^{-7}$ M, dexamethasone also causes a rapid decline in the levels of SP-A mRNA. The inhibitory effect of dexamethasone on SP-A mRNA levels is not dependent upon de novo mRNA and protein synthesis and, thus, appears to be mediated by a novel mechanism.

**MATERIALS AND METHODS**

*Organ Culture*—Lung tissues were obtained from human abortuses of 15-18 weeks of gestational age, in accordance with the Donors Anatomical Gift Act of the State of Texas. Consent procedures and protocols were approved by the Human Research Review Committee at the University of Texas Southwestern Medical Center at Dallas, TX. Lung tissues from 2 to 4 fetuses are commonly utilized for each experiment. The individual tissues were trimmed, rinsed, pooled, and then minced. Five to six explants were placed in each culture dish and maintained in organ culture in serum-free Waymouth’s MB752/1 medium (GIBCO), as described in detail previously (19). Lung explants were incubated either in control medium, in medium containing dexamethasone ($10^{-10}-10^{-5}$ M), Bt2cAMP (1 mM), or Bt2cAMP plus dexamethasone for up to 6 days. The dexamethasone was solubilized in ethanol; however, the concentration of ethanol in the culture medium never exceeded 0.01%, and this concentration of ethanol had no effect on the levels of SP-A mRNA in the fetal lung explants (data not shown). In some experiments, fetal lung tissues were incubated in medium that also contained either actinomycin D (5 µM) or cycloheximide (2 or 10 µg/ml) for time periods of up to 24 h. The media were changed daily.

*Isolation of RNA and Northern Blot Analysis of SP-A mRNA*—Total RNA was isolated from human fetal lung explants essentially by the method of Chingwin et al. (20) and subjected to Northern analysis using a 32P-labeled cDNA insert encoding rabbit SP-A (10), as described in detail previously (17). The blots were subjected to autoradiography using intensifying screens, and the relative amounts of SP-A mRNA were assessed by scanning densitometry of the autoradiograms.

*Isolation of Nuclei and Transcription Elongation Assay*—Isolation of nuclei and transcription elongation assays were performed as described previously using the rabbit SP-A cDNA as a hybridization probe (13). As a control for background, some filters either contained pUC18 DNA or no bound DNA. In both cases, the background radioactivity was found to be ≤10% of the values in control tissues. After the final wash step, the filters were subjected to autoradiography using intensifying screens, and the relative rates of transcription were assessed by one-dimensional scanning densitometry of the autoradiograms. Although this method can produce artifacts in the scanning of dot blots, in all cases the scanning beam was directed through the center of each dot. Furthermore, in some experiments the bound RNA was released by treating the filters with 0.25 ml of NaOH (0.4 M) for 30 min at room temperature and neutralized with 0.1 ml of acetic acid (1 M), and the radioactivity was determined by scintillation spectrometry. In those cases, the findings from densitometric scanning of autoradiograms and from elution of radiolabeled RNA from filters were found to be similar (data not shown).

**RESULTS**

*Effects of Dexamethasone in Various Concentrations and of Bt2cAMP on the Levels of SP-A mRNA and on the Transcriptional Activity of the SP-A Gene*—To define the mechanisms underlying the biphase effect of dexamethasone on SP-A gene expression in human fetal lung in vitro (17), transcriptional activity of the SP-A gene was analyzed in nuclei isolated from human fetal lung explants maintained for 5 days in control medium or in medium containing Bt2cAMP, in the absence or presence of dexamethasone at concentrations of $10^{-10}-10^{-5}$ M. Total RNA from the same tissues was analyzed for SP-A mRNA by Northern analysis. As shown in the autoradiogram of the Northern blot (Fig. 1A, upper panel) and the plot of the scanned autoradiogram (Fig. 1B, lower panel), a biphase effect of dexamethasone on the levels of SP-A mRNA was observed that was consistent with our previous observations (17). We were surprised to find, however, that dexamethasone had a dose-dependent stimulatory effect on SP-A gene transcription (Fig. 1A, center panel; Fig. 1B, upper panel). A maximal stimulatory effect of dexamethasone was apparent at a concentration of $10^{-7}$ M, with half-maximal stimulation occurring at concentrations between $10^{-8}$ and $10^{-6}$ M.

Bt2cAMP increased both the levels of SP-A mRNA and the transcriptional activity of the SP-A gene. In tissues incubated with Bt2cAMP and dexamethasone ($10^{-10}-10^{-5}$) in combination, dexamethasone at a concentration of $10^{-4}$ M increased the levels of SP-A mRNA over those of explants incubated with Bt2cAMP alone, whereas at $10^{-7}$ M, dexamethasone caused a marked reduction in the levels of SP-A mRNA (Fig. 1A, lower panel; Fig. 1B, lower panel). In contrast, in the same tissues, dexamethasone caused a dose-dependent increase in SP-A gene transcription; a stimulatory effect of dexamethasone was apparent at concentrations as low as $10^{-10}$ M. At concentrations of $10^{-8}-10^{-7}$ M, dexamethasone in the presence of Bt2cAMP caused an apparent synergistic increase in SP-A gene transcription (Fig. 1A, center panel; Fig. 1B, upper panel). The effect of dexamethasone to alter SP-A mRNA levels appeared to be specific; when the same Northern blots were probed using a radiolabeled actin cDNA insert, it was found that the levels of actin mRNA were unaffected by dexamethasone at all concentrations tested (data not shown). Also, the amounts of total RNA isolated from the explants were unaffected by dexamethasone treatment (data not shown).

*Effects of Dexamethasone on the Levels of SP-A mRNA and on SP-A Gene Transcription as a Function of Incubation Time*—To analyze the effects of dexamethasone on the levels of SP-A mRNA and on the transcriptional activity of the SP-A gene as a function of incubation time, human fetal lung explants were maintained in the absence or presence of Bt2cAMP for 5 days; dexamethasone ($10^{-7}$ M) was then added to the media, and the incubation was continued for 2, 6, 24, or 48 h. The levels of SP-A mRNA and the transcriptional activity of the SP-A gene in these tissues were analyzed by Northern blotting of total RNA and transcription elongation assays, respectively. As shown in the autoradiogram in Fig. 2A and the corresponding plot of the densitometric scan in Fig. 2B, the effect of Bt2cAMP on SP-A gene transcription appears to be relatively modest at the start (day 5 of incubation) of the time course experiment (1.3-fold as compared with control). After an additional 48 h of incubation, however, SP-A gene transcription in Bt2cAMP-treated tissues was increased to levels that were 5-fold greater than the corresponding control sample. Dexamethasone alone had no effect on
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Fig. 1. Effects of dexamethasone (Dex) in the absence or presence of Bt2cAMP (Bt2) on the transcriptional activity of the SP-A gene and on the levels of SP-A mRNA in human fetal lung in vitro. A, human fetal lung explants were maintained in culture for 5 days in the absence (C) or presence of dexamethasone (10^{-10} to 10^{-7} M) and in the absence or presence of Bt2cAMP (1 mM). Nuclei isolated from the tissues were subjected to transcription run-on analysis using the rabbit SP-A cDNA as a probe and an autoradiogram was obtained (center panels). Total RNA isolated from the same tissues was analyzed by Northern blotting using 32P-labeled rabbit SP-A cDNA as a probe and an autoradiogram was obtained (upper and lower panels). B, the autoradiograms in panel A were subjected to scanning densitometry, and the relative rates of SP-A mRNA degradation. To determine whether this effect of dexamethasone to cause a rapid decline in the levels of SP-A mRNA is dependent upon de novo RNA and protein synthesis, the effects of cycloheximide (2 or 10 μg/ml) and actinomycin D (5 μM) on the action of dexamethasone (10^{-7} M) to alter SP-A mRNA levels were studied in fetal lung tissues as a function of incubation time. We observed that actinomycin D at this concentration reduced RNA synthesis by 90% after 2 h and 74% after 24 h of incubation. Cycloheximide at 5 μg/ml inhibited protein synthesis by 66% after 2 h and 74% after 24 h of incubation; at 10 μg/ml, protein synthesis was inhibited by 87% at each of these time points.

In the experiment to test the effect of cycloheximide (2 μg/ml) on the dexamethasone-induced reduction of SP-A mRNA radiogram was obtained (center panels). Total RNA isolated from the same tissues was analyzed by Northern blotting using 32P-labeled rabbit SP-A cDNA as a probe and an autoradiogram was obtained (upper and lower panels). B, the autoradiograms in panel A were subjected to scanning densitometry, and the relative rates of SP-A gene transcription and mRNA levels of treated tissues are plotted relative to control values. When nonrecombinant pUC18 DNA was used in transcription elongation assays as a hybridization control, the background radioactivity was found to be ≤10% of control values (data not shown).
levels, the fetal lung explants were preincubated in medium containing Bt2cAMP for 5 days before the addition of dexamethasone and cycloheximide to induce SP-A mRNA to readily detectable levels. As shown in Fig. 3, dexamethasone (10^{-7} M) had a rapid effect in reducing the levels of SP-A mRNA in the Bt2cAMP-treated explants; the levels of SP-A mRNA were detectably reduced within 2 h of the addition of dexamethasone to the culture medium, and SP-A mRNA was reduced to barely detectable levels within 6–24 h of its addition to the medium. In tissues incubated with cycloheximide (2 μg/ml) alone the levels of SP-A mRNA remained relatively constant for 6 h and then declined. When fetal lung explants were incubated with cycloheximide and dexamethasone in combination, the levels of SP-A mRNA were unaffected after 2 h, but were markedly reduced after 4–24 h of incubation in a manner similar to that of tissues maintained in the presence of dexamethasone alone.

In consideration of the possibility that our failure to observe an effect of cycloheximide in blocking the action of dexamethasone to reduce SP-A mRNA levels was due to an incomplete inhibition of protein synthesis, studies were performed using cycloheximide at a concentration of 10 μg/mL. In the same experiment, the effects of actinomycin D (5 μM)
amethasone was evident at 6 h of incubation, and SP-A the same time point and were barely detectable after 24 h of also were investigated. In the study shown in Fig. 4, human retained.

block the inhibitory effect of dexamethasone on SP-A mRNA were markedly reduced after 24 h. Actinomycin D failed to rhhit SP-A cDNA as a probe, and an autoradiogram was obtained.

levels of SP-A mRNA after 6 and 24 h of levels of SP-A mRNA were lower than those observed after incubation with dexamethasone alone. In fetal lung explants incubated with cycloheximide (10 pg/ml) plus dexamethasone, the levels after 6 h of incubation; in fact, the levels of SP-A mRNA were reduced to barely detectable levels after 24 h. In lung explants incubated with actinomycin D alone, the levels of SP-A mRNA were reduced to barely detectable levels after 24 h. Actinomycin D failed to block the inhibitory effect of dexamethasone on SP-A mRNA levels after 24 h of incubation; in fact, the levels of SP-A mRNA were lower than those observed after incubation with dexamethasone alone. In fetal lung explants incubated for 6 h with cycloheximide (10 μg/ml) alone, the levels of SP-A mRNA were reduced as compared with untreated tissues at the same time point and were barely detectable after 24 h of treatment. In fact, the levels of SP-A mRNA in cycloheximide-treated fetal lung explants were quite similar to those observed after treatment with dexamethasone alone. In tissues incubated with cycloheximide plus dexamethasone, the levels of SP-A mRNA were similar to those observed after treatment with either factor alone. This marked reduction in SP-A mRNA levels after treatment with cycloheximide may be caused by its effect to inhibit both SP-A gene transcription and mRNA stability. In previous studies using fetal rabbit lung tissue in vitro (13), we observed that cycloheximide (2 μg/ml) had a rapid effect on inhibiting SP-A gene transcription in control explants, as well as the stimulatory effect of Bt,cAMP, suggesting that a labile protein factor is required for regulation of the transcriptional activity of the SP-A gene.

DISCUSSION

In the present study, we have observed that dexamethasone has a paradoxical effect on the expression of the gene encoding the major surfactant-associated protein, SP-A, in human fetal lung in vitro. The glucocorticoid caused a dose-dependent stimulation of SP-A gene transcription, with a maximum stimulatory effect evident at concentrations of 10⁻⁹–10⁻⁷ M. At these concentrations, dexamethasone acted synergistically with Bt,cAMP to increase the rate of SP-A gene transcription. On the other hand, dexamethasone caused a marked reduction in the levels of SP-A mRNA and reduced the magnitude of the stimulatory effect of Bt,cAMP on SP-A mRNA levels. The effect of dexamethasone (10⁻⁷ M) to reduce SP-A mRNA levels was not blocked by inhibitors of mRNA and protein synthesis. In preliminary studies, we observed that in fetal lung explants incubated with actinomycin D and dexamethasone (10⁻⁷ M) in combination, the rate of decline of SP-A mRNA levels was >3 times greater than that observed in explants incubated in the presence of actinomycin D alone. From all of these findings, it is apparent that the effect of dexamethasone (>10⁻⁸ M) to reduce SP-A mRNA levels is not mediated by an inhibitory effect of the steroid on SP-A gene transcription. Rather, it appears that dexamethasone at such concentrations is eliciting its effects by increasing the rate of degradation of SP-A mRNA. The present findings are suggestive that the stimulatory effect of dexamethasone on SP-A mRNA levels at concentrations of 10⁻¹⁰–10⁻⁹ M reflect effects of the steroid to increase SP-A gene transcription in the absence of an effect to decrease the stability of SP-A mRNA. At concentrations >10⁻⁸ M, however, the action of the glucocorticoid to increase the rate of SP-A mRNA degradation overrides its stimulatory effect on SP-A gene transcription, resulting in decreased levels of SP-A mRNA. Although the majority of studies concerning the control of eucaryotic gene expression have been directed toward the regulation of gene transcription, it has become increasingly apparent that post-transcriptional mechanisms serve an equally important role in the regulation of mRNA levels (21). Such post-transcriptional events as mRNA processing and transport out of the nucleus, as well as mRNA degradation, and stabilization, have been reported to be regulated by hormones and cellular and environmental factors (22). Modulation of mRNA stability may provide an important homeostatic mechanism for the regulation of synthesis of a number of cellular proteins. Iron has been reported to negatively regulate the levels of mRNA for the human transferrin receptor; these effects on transferrin receptor mRNA levels are primarily due to a specific decrease in transferrin receptor mRNA stability (23). The iron-dependent regulation of transferrin receptor mRNA levels requires the presence of a stem-loop structure within the 3' untranslated region of the mRNA (23, 24) and is inhibited by actinomycin D or cycloheximide, suggesting that an iron-regulated RNase or other protein with a relatively short half-life binds to this structure and mediates transferrin receptor mRNA degradation (23).

A number of hormones and second messengers have been reported to exert effects on gene expression by altering the stability of specific mRNAs. Glucocorticoids selectively inhibit the transcription of the interleukin-1β gene and cause a marked decrease in the stability of its mRNA (25). This effect is blocked either by actinomycin D or cycloheximide (25), suggesting that a glucocorticoid-inducible protein may specifically act to reduce interleukin-1β mRNA stability. The mRNAs for interleukin-1β and a number of other proinflammatory mediators contain within their 3' untranslated regions one or more copies of the sequence AUUUA, which is believed to mediate their selective degradation and resultant short half-life (26, 27). Although one

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might suggest that glucocorticoids increase interleukin-1β degradation by inducing the synthesis of an RNase or other protein that binds selectively to this A + U-rich sequence, in the same cell it was found that dexamethasone had no effect on the stability of 40S mRNA, which contains several copies of such an A + U-rich sequence (25). Glucocorticoids also have been reported to reduce the levels of mRNAs for interferon (28) and granulocyte-macrophage colony-stimulating factor (29) in a variety of target cells, while having little effect on transcription of the respective genes.

Although the mechanisms whereby hormones and factors regulate the stability of specific mRNAs must differ significantly, it appears that in all reported examples the induction of mRNA destabilization is dependent upon ongoing protein synthesis, suggestive of the induction of protein factors that mediate mRNA degradation. In the present study, however, it was found that the effect of dexamethasone (10⁻⁷ M) to cause a rapid decrease in the levels of SP-A mRNA was independent of ongoing mRNA and protein synthesis. In unpublished studies, we found that the effect of glucocorticoids to reduce SP-A mRNA levels is rapidly reversible. Based on these observations, we suggest that glucocorticoids may effect a decrease in SP-A mRNA stability by inhibiting the synthesis of a labile protein(s) which is required for SP-A mRNA stability. This hypothesis is supported by the finding that in fetal lung explants incubated with cycloheximide (10 μg/ml) for 6 or 24 h, the levels of SP-A mRNA were reduced to levels comparable with those of explants incubated for these periods with dexamethasone (10⁻⁷ M) alone (Fig. 4). It has recently been suggested that glucocorticoids decrease the stability of 3-hydroxymethylglutaryl-CoA reductase mRNA in rat liver tissue by counteracting a thyroid hormone-induced protein which stabilizes this mRNA species (30). Alternatively, dexamethasone may induce the post-translational modification of a stable protein factor resulting in an increase in its capacity to facilitate SP-A mRNA degradation.

The mechanisms underlying the biphasic effect of glucocorticoids on the levels of SP-A mRNA have not been defined. Possible explanations for the apparent biphasic effect could be differences in the sensitivities to dexamethasone or the mRNA stability. In studies of Fu5-5 cells infected with mouse mammary tumor virus (31). It also has been reported that glucocorticoid induction of tyrosine aminotransferase to the culture medium when lung explants are pretreated with Bt,cAMP (Fig. 2).

It is important to note that this action of glucocorticoids to reduce SP-A mRNA stability appears to be unique to human fetal lung tissue. In studies with fetal rat lung tissue in vitro, we have observed that glucocorticoids cause a transient decrease in the levels of SP-A mRNA followed by a subsequent induction of SP-A mRNA levels with increased time of incubation; however, these effects of glucocorticoids on SP-A expression in fetal rat lung tissue appear to be regulated primarily at the transcriptional level (13). In fetal rat lung tissue, glucocorticoids have been reported to increase the levels of SP-A mRNA when administered in vivo (15).

Another major finding of the present study is the synergistic action of dexamethasone and Bt,cAMP to increase SP-A gene transcription. As shown in Fig. 2, when fetal lung explants were incubated for 48 h in the presence of either dexamethasone (10⁻⁷ M) or Bt,cAMP, SP-A gene transcription was increased 1.4- and 6-fold, respectively. On the other hand, a >15-fold increase in SP-A gene transcription was detected in explants incubated for the same period in medium containing Bt,cAMP and dexamethasone in combination. In fetal lung explants incubated for 5 days in the presence of Bt,cAMP and dexamethasone in combination, a synergistic effect of these agents on SP-A gene transcription was observed at concentrations of dexamethasone of 10⁻⁷ to 10⁻⁵ M (Fig. 1).

Glucocorticoids and cyclic AMP analogues have been found to have either additive or synergistic effects on the induction of tyrosine aminotransferase mRNA in liver tissue (34) and in hepatoma cell lines (35). Although it is clear that cyclic AMP is acting to increase tyrosine aminotransferase mRNA levels, at least in part, at the transcriptional level (34), the molecular mechanisms for the combined effects of cyclic AMP and glucocorticoids on tyrosine aminotransferase gene expression have not been determined.

The actions of glucocorticoids to enhance eucaryotic gene transcription appear to be mediated by the interaction of the glucocorticoid receptor with specific sequences within the 5'-flanking regions of responsive genes (see Ref. 36 for review). Cyclic AMP is believed to mediate an increase in gene expression through the binding of a specific nuclear phosphoprotein to conserved cyclic AMP regulatory elements within the 5'-flanking regions of responsive genes (37). It will be of great interest to define the mechanisms whereby cyclic AMP and glucocorticoids act to increase SP-A gene transcription, as well as the molecular interactions that result in the synergistic effects of these factors to activate transcription of this gene.

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