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Cyclic AMP-dependent protein kinase (PKA) gene expression is developmentally regulated in fetal lung

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Abstract

We characterized the ontogeny of cAMP-dependent protein kinase (PKA) enzymatic activity and PKA subunit mRNA expression in developing lung. The lungs of fetal Sprague–Dawley rat pups were removed after 16, 18, or 20 days of gestation and at term. PKA activity was greatest in the 18- and 20-day gestation lungs. Tissue cAMP levels were lowest in the 16-day lungs and increased with lung maturity. We were able to detect only low levels of mRNA for the Cβ subunit of PKA by northern blot analysis of total lung RNA and we were able to detect mRNA for the RIβ and RIIβ subunits only by RT-PCR. Therefore, we limited our analysis of PKA subunit mRNA levels to those for Cα, RIα and RIIα. The mRNA levels for Cα, were highest in the 16-day lung, decreased at 18 and 20 days, were lower in the newborn and lowest in the adult lung. RIα mRNA levels were also highest at 16 days and lowest in the adult lung. However, RIIα mRNA levels were similar in the 18-day, 20-day and newborn lungs. Dexamethasone treatment of fetal lung explants resulted in a small decrease in RIα mRNA levels but was not associated with a change in PKA activity. We conclude that PKA activity and PKA subunit mRNA expression are developmentally regulated in fetal lung. Such regulation results in optimal PKA activity at the time of type II alveolar cell differentiation, presumably in preparation for air breathing. The absence of an effect of glucocorticoid on PKA activity suggests that glucocorticoids are not responsible for the increase in PKA activity which accompanies this critical time in lung maturation. © 1998 Elsevier Science B.V.

Keywords: cyclic AMP-dependent protein kinase; Lung development; cyclic AMP; Fetal lung; mRNA

1. Introduction

Cyclic adenosine monophosphate (cAMP) and factors which increase intracellular cAMP levels have been demonstrated to promote lung development and alveolar type II cell differentiation in vivo and in vitro. Barrett et al. [1] demonstrated that treatment of pregnant rabbits with aminophylline resulted in an increase in fetal lung cAMP levels and an associated increase in [^{14}C]-choline incorporation into surfactant specific disaturated phosphatidylcholine. β-adrenergic agonists, which activate adenylyl cyclase and increase intracellular cAMP, increase pulmonary surfactant production and secretion in adult rats [2] and fetal sheep [3]. The treatment of perfused adult rat lungs with the cAMP analog, 8-Br-cAMP, results in an increase in alveolar levels of the type II cell specific, surfactant-associated protein, SP-A, and an associated increase in tissue levels of SP-A mRNA [4]. We have previously reported that prostaglandins stimulate alveolar type II cell differentiation in hu-

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man fetal lung in vitro through an increase in tissue production of cAMP [5]. The direct effect of cAMP analogs to accelerate type II cell differentiation and to stimulate production of the surfactant-associated protein, SP-A, in rat fetal lung [6] and human fetal lung [7] in culture has also been demonstrated.

Cyclic AMP is a ubiquitous regulatory molecule important for the induction of transcription of many eukaryotic genes [8]. The downstream actions of cAMP are dependent upon its binding to cAMP-dependent protein kinase (PKA) [9]. PKA is a tetrameric holoenzyme composed of two regulatory (R) and two catalytic (C) subunits which dissociate upon binding cAMP into an R-subunit dimer and two active C-subunits [10]. There are two types of PKA holoenzymes, type I and type II, each distinguished by its type I (RI) or type II (RII) regulatory subunit. The primary function of the regulatory subunit is to bind and inactivate the catalytic subunit in the absence of cAMP [11]. Two isoforms of each regulatory subunit, designated RIα, RIβ, RIIα and RIIβ, have been identified and cloned. RIα [12] and RIIα [13] are expressed in most cells, whereas the expression of RIβ [14] and RIIβ [15] is more limited. The type II holoenzyme has been reported by Giembycz and Diamond to be the dominant form of PKA in adult guinea pig lung, however, the subtype of the R subunits were not determined [16]. The specific regulatory subunit isoforms present in developing lung have not been determined. Three isoforms of the C subunit, designated Cα, Cβ, and Cγ, have also been described [17]. The Cα and Cβ isoforms are expressed in adult human lung, whereas the Cγ isoform is expressed only in the testis [17].

The objective of the current investigation was to test the hypothesis that PKA activity and PKA subunit mRNA expression is developmentally regulated in lung tissue. We characterized the ontogeny of the cAMP second messenger system in developing rat lung by determining PKA activity, mRNA levels for the PKA subunits, Cα, RIα and RIIα, and tissue cAMP levels in whole homogenates of fetal, neonatal and adult rat lung. Though we detected only low levels of Cβ mRNA by northern blot analysis and were able to detect RIβ and RIIβ mRNA only by RT-PCR, we found that mRNA levels for the other PKA subunits changed during development in a pattern consistent with changes in PKA activity. A number of factors including glucocorticoids, epidermal growth factor, prostaglandins, cAMP analogs and thyroid hormone are known to stimulate fetal lung maturation in vivo and in vitro [18–20]. Of these factors, glucocorticoids are frequently used clinically to induce maturation of the human fetal lung. Therefore, it was also of interest to determine if PKA activity and PKA subunit mRNA levels are regulated by the synthetic glucocorticoid, dexamethasone, in rat fetal lung explants maintained in vitro.

2. Materials and methods

2.1. Tissues for study and organ culture

Fetal, neonatal and adult lung tissue from timed-pregnant Sprague–Dawley rats (Sasco, Omaha, NE) were harvested, flash frozen in liquid N_2 and stored at -70°C for later processing. For studies involving the in vitro effects of dexamethasone on steady state levels of PKA subunit mRNA, 18-day gestation rat fetal lung tissues were harvested and placed in explant culture as previously described for human fetal lung explants [21]. Briefly, fetal lung tissues were dissected free of major blood vessels and airways, minced into 1–2-mm^3 pieces with a sterile scalpel blade and placed in organ culture. Cultures were maintained for 48 h in serum-free Waymouth's MB752/1 medium, (GIBCO, Grand Island, NY) at 37°C in the absence or presence of dexamethasone (100 nM). The media was replaced after 24 h of incubation. Pooled lung tissues from one or two litters were used for each experiment.

2.2. Assay of cAMP-dependent protein kinase activity

Fetal lung tissues for PKA activity assays were harvested as above or, if maintained in vitro, tissues were harvested after 48 h of incubation then frozen in liquid N_2 and stored at -70°C until assays were performed. Tissues were homogenized in 0.5 ml of 10 mM $NaPO_4$ (pH 7.0), 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM isobutylmethyl xanthine, and 0.1 mM phenylmethylsulfonyl fluoride and centrifuged for 15 min at $12\,000 \times g$. The supernatant was collected and a protein determination by the method of Bradford [22] was performed. The supernatant was assayed for PKA activity as described by

Roskoski [23] and modified by Howard et al. [24]. Reaction mixtures containing 10 μg of supernatant protein, 0.1 mM [^{32}P]ATP (200 cpm/pmol) and 20 μM kemptide (Sigma Chemical, St. Louis, MO) in a total volume of 50 μl 25 mM Tris–HCl (pH 7.4) buffer were incubated at 30°C for 5 min. Next, 20 μl of reaction mixture was spotted onto phosphocellulose paper (Whatman P-81) and the papers were washed four times in 75 mM phosphoric acid. Radioactivity was then determined by scintillation spectrometry. Assays were performed in duplicate or triplicate for each incubation condition within each experiment. Data are expressed as the rate of phosphorylation of the target peptide (kemptide) per microgram of protein assayed (pmol/min/μg protein). Each experiment was performed three to six times.

2.3. RNA isolation and analysis

Total RNA was isolated by the method of Chirgwin et al. [25] and quantitated by determining the absorbance at 260 nm. Ten micrograms of total RNA for each sample was separated on a 1.2% agarose/formaldehyde gel then transferred by vacuum blotting (Bio-Rad Laboratories, Hercules, CA) to a Nytran membrane (Schleicher and Schuell, Keene, NH) for northern blot analysis. The agarose gels were stained with ethidium bromide to verify complete transfer of RNA to the membranes. The RNA was cross-linked to membranes by irradiation with a hand-held UV light source for 5 min then baked at 80°C for 90 min. Membranes were prehybridized at 42°C for 6–12 h in hybridization buffer [BSA (0.2% wt/vol), polyvinylpyrrolidone (0.2%, wt/vol), ficoll (0.2%, wt/vol), Tris–HCl (50 mM, pH 7.4), Na pyrophosphate (0.1%, wt/vol), sodium dodecyl sulfate (1%, wt/vol), formamide (50%, vol/vol), dextran sulfate (10%, wt/vol), NaCl (1 M), and denatured salmon sperm DNA (100 μg/ml)]. The membranes were hybridized at 42°C overnight in hybridization buffer containing 1×10^6 cpm/ml of ^{32}P-labelled cDNA specific for rat Cα, RIα, or RIIα mRNA (cDNAs were kind gifts of Dr. Richard Maurer, Oregon Health Sciences University, Portland, OR) [26]. After hybridization, the blots were washed twice for 5 min at room temperature in 2 × SSC [NaCl (300 mM), sodium citrate (30 mM), pH 7.0], twice for 30 min at 42°C in 1 × SSC plus sodium dodecyl sulfate (1%, wt/vol), and once for 15 min at room temperature in 0.1 × SSC. Blots were exposed to X-ray film with an intensifier screen at −70°C for 24–72 h. Blots were stripped and reprobed with a ^{32}P-labelled cDNA specific 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. Plotted values for the Cα, RIα and RIIα mRNA levels during gestation (Fig. 3B) were normalized to the levels determined for the 'Adult' lung.

2.4. Cyclic AMP assays

Whole rat lung tissues which had been flash frozen were homogenized in 0.5 ml of buffer containing 0.1 M $NaPO_4$ (pH 7.4), 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride and 0.1 mM isobutylmethyl xanthine. An aliquot (20 μl) of each homogenate was removed for analysis of protein content by the method of Bradford [22] and another aliquot (300 μl) was boiled in a water bath for 10 min. The boiled homogenates were then centrifuged for 15 min at 12 000 × *g*. Cyclic AMP levels were determined in the supernatants by radioimmunoassay with a commercially available kit (Amersham, Arlington Heights, IL). Cyclic AMP levels were determined in triplicate for each sample and values are expressed as pmol of cAMP/mg of tissue protein. Determinations of tissue cAMP content were made on four different sets of tissues.

2.5. Reverse transcription–polymerase chain reaction

Total RNA isolated from whole rat lung or adult rat brain was incubated with oligo-dt primer (Promega, Madison, WI) and avian myeloblastosis virus reverse transcriptase (Promega) to generate first strand cDNA for polymerase chain reaction (PCR) amplification of RIβ, RIIβ and β-actin cDNA. PCR conditions were optimized for each set of primers with a PRC Optimizer™ kit (Invitrogen, San Diego, CA). Reaction mixtures of 50 μl total volume containing 10 μl 5× sample buffer (PCR Optimizer™ kit, buffer 'G' for RIβ, buffer 'C' for RIIβ, and buffer 'N' for β-actin), 5 μl dimethyl sulfoxide, specific

primers, dNTPs, Taq DNA polymerase (Boehringer Mannheim), and H_2O were amplified for 35 cycles after preheating the PCR reaction mixtures to 94°C for 2 min. For amplification of RIβ cDNA, a forward primer 5′-CTACTGTGAAGGCCAAAACG-3′, and reverse primer 5′-CAGGATCTCAGAGCAGGGG-3′ generating a 461 bp product were employed with each cycle being 94°C for 1 min, annealing at 49°C for 1 min, and 72°C for 1 min. For amplification of RIIβ, a forward primer 5′-TGGTGCTCTGTGGGGTTT-3′, and reverse primer 5′-ATAATTTCCATGCAAGGTCCC-3′ generating a 453 bp product were employed with each cycle as above except for an annealing temperature of 51°C for 1 min. For amplification of β-actin, commercially available primers (Research Genetics, Huntsville, AL) were employed yielding a 289 bp product. The RT-PCR was performed on three separate sets of total RNA with similar results from each set. Total RNA from adult rat brain served as a positive control.

2.6. Statistical analysis

Statistical comparisons of the densitometric mRNA data were made using a two-tailed Dunnett's test [27]. Other data were analyzed by one way ANOVA. Comparisons between individual groups were made with the paired *t*-test as appropriate. Differences were considered significant when $P < 0.05$.

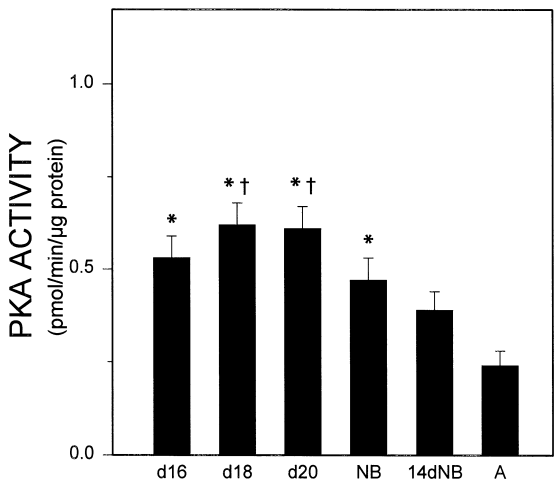

Fig. 1. PKA activity in homogenates of whole lung from Sprague–Dawley rats of 16-, 18-, 20-day gestation, term newborn (NB), 14-day neonate (14 dNB) and adult (A). Values represent mean ± SEM ($n = 5$), $*$ $P < 0.05$ vs. adult (A), †$P <$ 0.05 vs. 14 dNB.

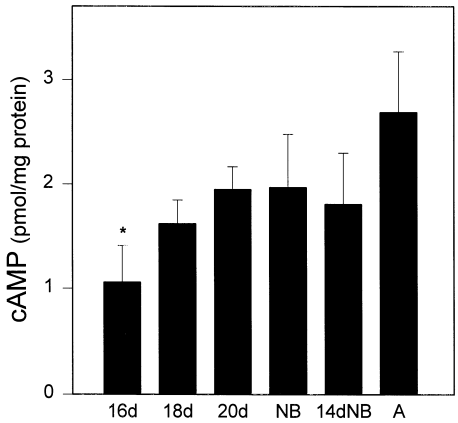

Fig. 2. Tissue levels of cAMP in whole lung tissues from Sprague–Dawley rats of 16-, 18-, 20-day gestation, term newborn (NB), 14-day neonate (14 dNB) and adult (A). Values represent mean ± SEM ($n = 4$), $*$ $P = 0.07$ vs. adult (A).

3. Results

3.1. PKA activity in developing rat lung

PKA activity was determined in homogenates of whole lung from Sprague–Dawley rats of 16-, 18-, 20-day gestation, term newborn (22 day gestation), 14-day neonatal and adult (Fig. 1). PKA activity was greatest in the 18-day and 20-day gestation fetal lung

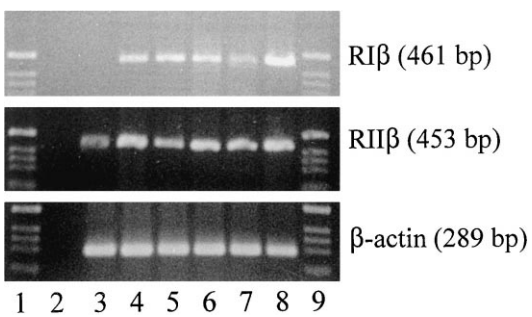

Fig. 3. Presence of RIβ and RIIβ mRNA in developing rat lung detected by RT-PCR. Total RNA from fetal, newborn and adult rat lung was amplified by RT-PCR as demonstrated on this representative ethidium bromide-stained agarose gel. Lane 1: DNA ladder; Lane 2: H_2O (negative control); Lane 3: 16-day fetal lung; Lane 4: 18-day fetal lung; Lane 5: 20-day fetal lung; Lane 6: newborn lung; Lane 7: adult lung; Lane 8: adult rat brain (positive control); Lane 9: DNA ladder.

and was significantly higher in fetal lung at all stages examined and in the newborn lung when compared to the adult lung. These findings suggest the developmental regulation of PKA activity with the greatest activity occurring in late gestation, perhaps, in preparation of neonatal life. Immunostaining was performed for the Cα and RIα subunits to determine if cell-type specific changes in the expression of these subunits might occur during lung development in association with our observed changes in PKA activity. However, Cα and RIα subunits were found to be evenly distributed across all cell types throughout development (data not shown) (Jeanne M. Snyder, personal communication).

As demonstrated in Fig. 2, cAMP levels also changed in association with rat lung development. Cyclic AMP levels were lowest in the 16-day fetal lungs and increased with lung maturation.

3.2. PKA mRNA levels in developing lung

We initially attempted to study the ontogeny of mRNA levels for both the α and β subunits for C, RI and RII, however, we were able to detect only low levels of mRNA for Cβ and we were unable to detect mRNA for RIβ and RIIβ by northern blot analysis of total lung RNA. Therefore, we performed RT-PCR to determine if RIβ and RIIβ subunit

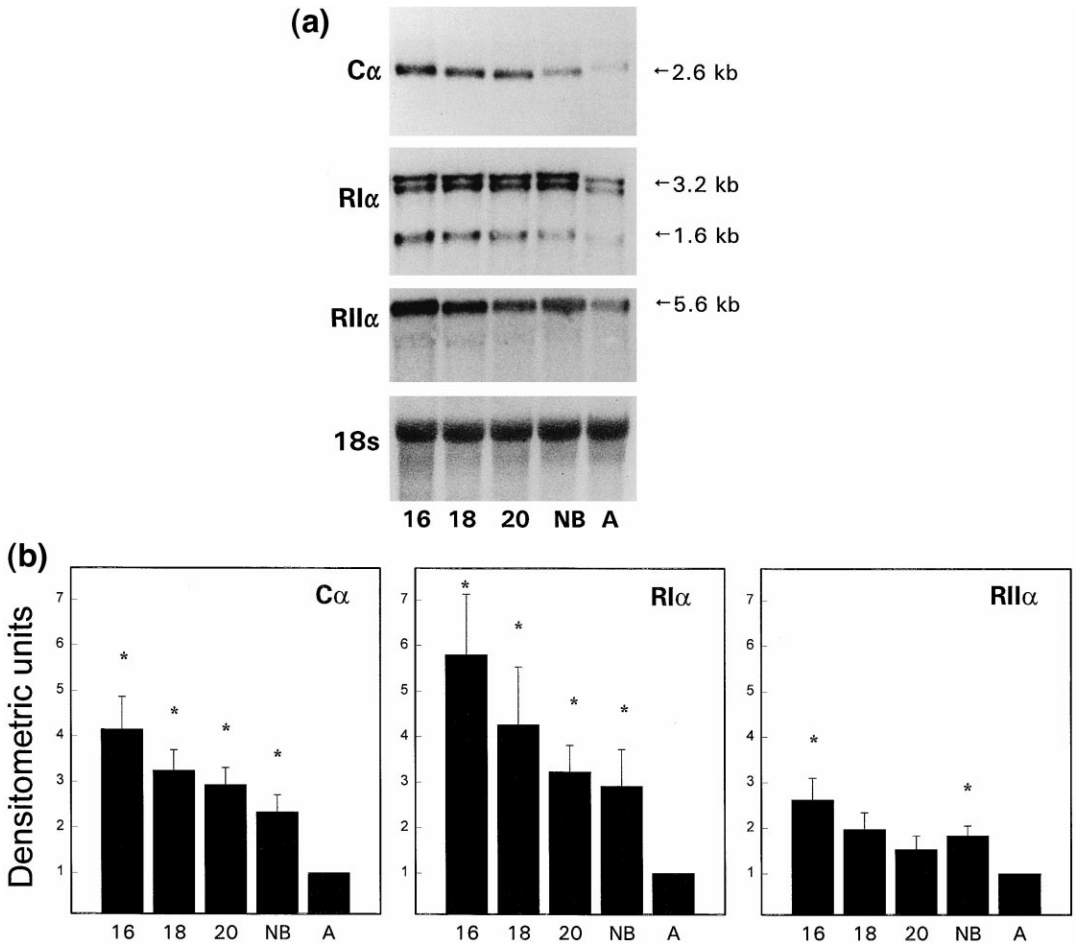

Fig. 4. Northern blot analysis of PKA subunit mRNAs. Total RNA (10 μg) from the lungs of 16-, 18-, 20-day gestation, term newborn (NB), and adult (A) Sprague–Dawley rats was analyzed by northern blots using labelled cDNAs specific for rat Cα, RIα, RIIα. (A) A representative autoradiogram. (B) Relative levels of mRNA for PKA subunits determined by scanning densitometry and corrected for loading and transfer errors with desitometric values for 18S rRNA. Values are expressed as mean $\pm$ SEM ($n = 3$–5). $*$ $P < 0.05$ compared to adult (A) condition.

Table 1
Effect of regulatory factors on PKA subunit mRNA in rat fetal lung in vitro

	Cα mRNA	RIα mRNA	SP-A mRNA
Control	1.00	1.00	1.00
Dex (100 nM)	0.91 ± 0.14	0.81 ± 0.06 *	1.38 ± 0.06 *

Explants of 18-day rat fetal lung were placed in culture for 48 h in the absence or presence of dexamethasone (Dex). Densitometric values are relative to mRNA levels in the control tissues. Values are expressed as the mean $\pm$ SEM for three independent experiments. * $P < 0.05$ vs. control.

mRNA are expressed in the developing rat lung (Fig. 3). RIβ mRNA was undetectable at 16 days of gestation but was detectable at 18 days of gestation and later. In contrast, RIIβ mRNA was detectable by RT-PCR in the fetal and adult lung at 16 days of gestation and at all other ages which were assessed (Fig. 3). Because of the difficulty in detecting and quantitating the PKA β-subunit mRNAs, we limited further analysis of PKA subunit mRNA levels to those for Cα, RIα and RIIα. We found that mRNA levels for rat Cα were highest in 16-day fetal rat lung and decreased through gestation (Fig. 4A and B). However, Cα mRNA levels were significantly higher in fetal and newborn lung tissues than in adult rat lung tissues. Cα mRNA levels in 16-day fetal lung were four-fold greater and newborn levels were two-fold greater than levels in adult rat lung (Fig. 4B). Northern blotting for RIα yielded bands at 1.6 kb and a doublet at 3.0/3.2 kb (Fig. 4A). The multiple bands are known to be products of alternative polyadenylation site signalling [28]. Analysis of several blots revealed little variation in the 3.0/3.2 kb doublet with development, with the exception of the adult lung for which the 3.0/3.2 doublet mRNA was approximately 50% of the level observed at all other developmental time points. However, there were significant changes in the RIα 1.6 kb mRNA signal with gestation and these were quantitated (Fig. 4B). RIα mRNA (1.6 kb) levels in 16-day fetal lung were nearly six-fold greater, and newborn levels were three-fold greater, than levels in adult rat lung (Fig. 4B). In contrast, RIIα mRNA levels, though higher in 16-day fetal and newborn lung compared to the adult lung, were not significantly different in 18- and 20-day fetal lungs when compared to the adult (Fig. 4). Together, these data demonstrate that PKA mRNA subunit levels are developmentally regulated in rat lung.

3.3. Effect of glucocorticoid on PKA subunit mRNA levels in rat fetal lung in vitro

In order to determine if glucocorticoids might affect steady state levels of PKA subunit mRNAs, we incubated rat fetal lung explants in the absence or presence of dexamethasone (100 nM). This concentration of dexamethasone has previously been demonstrated to influence tissue morphology, surfactant protein levels and surfactant phospholipid production in fetal lung in vitro [18]. As shown in Table 1, we found that the steady state levels of mRNA for Cα were not changed by 48 h of incubation in the presence dexamethasone. However, RIα mRNA levels were significantly decreased by 19% compared to levels in untreated (control) tissues (Table 1). Steady state levels of mRNA for the surfactant associated protein, SP-A, were determined to serve as a positive control. SP-A mRNA levels were increased in the dexamethasone treated tissues as previously reported [6].

In order to determine if the effect of hormone treatment on RIα mRNA levels is associated with changes in PKA activity in fetal lung explants, we

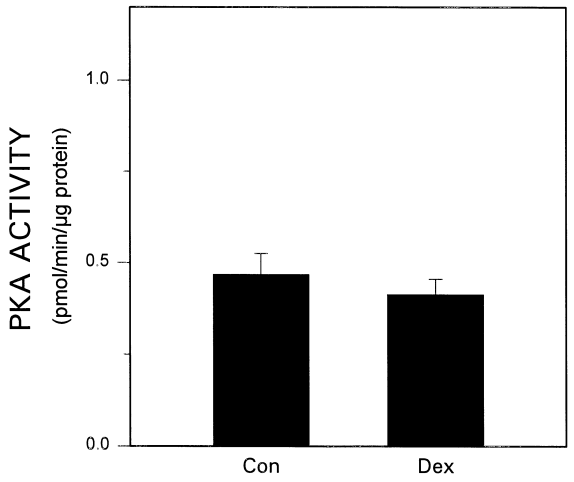

Fig. 5. Effect of dexamethasone on PKA activity. Rat fetal lung explants were incubated for 48 h in the absence (Con) or presence of dexamethasone (Dex). PKA activity was then determined. Values represent the mean $\pm$ SEM for four independent experiments performed in duplicate. There was no significant difference between the two conditions.

determined PKA activity in explants after 48 h of exposure to dexamethasone (Fig. 5). PKA activity was unchanged in tissues treated with dexamethasone. PKA activity assays on tissues incubated in the absence or presence of dexamethasone for 0, 0.5, 2, 4, 8, 12, 24, 36, and 48 h also failed to demonstrate significant changes in PKA activity associated with glucocorticoid treatment (data not shown).

4. Discussion

Cyclic AMP-dependent protein kinase mediates the intracellular actions of cAMP via phosphorylation of specific target proteins. Whitsett et al. [29] have previously demonstrated ontogenic changes in cAMP enhanced phosphorylation by PKA of cytosolic proteins in the lungs of developing rats. The activation of PKA by terbutaline and forskolin in isolated type II cells with an associated secretion of phosphatidylcholine has also been demonstrated by Rice et al. [30]. The importance of the PKA mediated effects of cAMP in developing lung have been further studied by Ballard et al. [31], who found that mRNA levels for the surfactant associated proteins, SP-A and SP-B, decrease in human fetal lung explants treated with H-8, an inhibitor of PKA activity. These studies demonstrate the direct participation of PKA in cAMP regulated events in the developing lung and in type II alveolar cells.

In the present study, we defined the ontogeny of PKA activity, tissue cAMP levels and PKA subunit mRNA levels during rat lung development. We found that PKA activity was greatest in 18- and 20-day fetal lung, the period of rat alveolar type II cell differentiation [32], and decreased in the 14-day newborn and adult lung. Importantly, our finding of maximal PKA activity in the 20-day whole fetal lung is similar to previous data demonstrating maximal endogenous PKA activity in isolated rat type II cells at 20 days of gestation [33]. Consistent with our finding of decreased PKA activity in the adult lung, cAMP levels, which have been demonstrated by Richardson et al. [26] to down-regulate PKA activity via degradation of Cα protein, were lowest in the early gestation lung and increased with lung maturity. In contrast to cAMP levels, but similar to PKA activity, mRNA levels for Cα, and RIα decreased through gestation and were significantly lower in the adult lung compared to all earlier periods. RIIα mRNA levels, however, varied to a lesser degree during gestation than did the Cα and RIα mRNA levels but were still significantly greater in the newborn lung than in the adult lung. These data demonstrate the developmental regulation of the cAMP/PKA system in rat lung. The inverse relationship between cAMP levels and PKA activity in later development supports the possibility that cAMP may negatively regulate PKA activity in the developing lung as it does in tumor cell lines [26]. However, as the effect of cAMP on PKA activity occurs via a post-transcriptional mechanism, changes in cAMP levels with lung development do not account for the observed changes in PKA subunit mRNA levels. These changes may occur secondary to unidentified hormonal modulation, or, perhaps to changes in the cell types which produce these mRNAs. Observing changes in the cellular distribution of PKA subunits is difficult as PKA is a ubiquitous enzyme. Immunohistochemical studies of the localization of the Cα and RIα subunits of PKA have shown that they are evenly distributed in developing rat fetal lung among all cell types (Jeanne M. Snyder, personal communication).

Though it appears that we have demonstrated only modest changes in PKA activity associated with maturation of the rat lung, there is precedence in the literature to support the contention that these observed changes may indeed be physiologically important. For example, increases in PKA activity of 40% induced by dibutyryl-cAMP (1 mM) are associated with the induction of morphological changes consistent with differentiation of schwann cells in vitro [34]. We observed endogenous PKA activity in both the 18-day and 20-day fetal lung to be approximately 30% and 55% higher than the PKA activity observed in the newborn and 14-day newborn lung, respectively (Fig. 1). This degree of difference in PKA activity is similar to the 48% increase in PKA activity observed in isolated rat type II cells incubated in the presence of dibutyryl-cAMP (1 mM) [33]. The ability of this degree of change in PKA activity to effect changes in the developing lung has been demonstrated in models of rat, rabbit and human fetal lung development. For example, the incubation of rat fetal lung explants in the presence of dibutyryl-cAMP (200 μM) stimulates choline incorporation into phos-

phatidylcholine [6], and the incubation of rabbit fetal lung explants [35] and human fetal lung explants [7] in the presence of dibutyryl-cAMP (1 mM) stimulates increased levels of SP-A, and SP-A mRNA. Dibutyryl-cAMP (1 mM) also accelerates type II cell differentiation and morphological change in human fetal lung in vitro [7]. Taken together, these data suggest that the changes in endogenous PKA activity that we observed with rat lung development may indeed be adequate to be responsible for physiological changes in the developing lung.

Hormonal effects on PKA mRNA levels in the developing lung have not been previously reported. However, the hormonal regulation of PKA subunit mRNA levels in other systems has been described previously. Kurten et al. [36] reported that the in vitro differentiation of preadipocytes to adipocytes is associated with induction of the PKA subunits RIα, RIIβ and Cα. Dexamethasone treatment of the adipocytes resulted in decreased levels of mRNA for Cα, RIα and RIIβ, whereas RIIβ and Cα mRNA were induced by the treatment of cultures with indomethacin, an inhibitor of prostaglandin synthesis [36]. Basal levels of RIα protein have been reported to be responsible for the maintenance of cellular PKA activity. Jones et al. [37] found human hepatoma cells to contain only 10% of the RIα mRNA present in other cultured cell types. This decrease in RIα mRNA resulted in increased basal activity of PKA which, they speculated, results in PKA mediated constitutive hyperphosphorylation of CREB [37]. CREB (CRE-binding protein) is a specific nuclear transcription factor required for cAMP induction and basal level expression of CRE-containing genes [38,39]. We did not find a significant effect of dexamethasone on Cα mRNA levels in rat fetal lung tissues maintained in vitro. In contrast, RIα mRNA levels were significantly decreased in tissues treated with dexamethasone. The fact that we did not observe a change in PKA activity in association with a decrease in RIα mRNA levels may be the result of the relatively modest decrease in RIα mRNA levels (19%) that we observed. Thus, it is unlikely that this modest change in RIα mRNA levels in response to glucocorticoids is of physiological significance.

Endogenous PKA activity is determined by intracellular cAMP levels, the relative amounts of regulatory and catalytic subunits, and the actions of endogenous PKA inhibitors such as the thermostable protein inhibitor of PKA, PKI [40]. The complexity of the system increases when one considers the existence of one or more isoforms for each of the PKA subunits and for PKI [41]. Our finding of increased endogenous PKA activity in the developing lung at 18 and 20 days of gestation suggests that all of the biochemical entities involved in determining PKA activity are regulated in a fashion which optimizes the response to cAMP in preparation for extrauterine life and air breathing. The results of our investigation suggest the developmental control of some or all of the factors which regulate PKA activity. Our in vitro findings of no change in PKA activity with dexamethasone treatment in spite of a decrease in steady state levels of mRNA for an important regulatory subunit (RIα) supports the notion that cAMP action in the developing lung occurs independently of the influence of glucocorticoids. The lack of influence by glucocorticoids on PKA activity does not rule out the possibility that our observed changes in PKA activity and PKA mRNA expression are modulated by other, as yet unidentified, factors in the developing lung. We speculate that further work to determine the responsible regulatory factors and the mechanisms, whereby their effects may permit one to pharmacologically modulate the response of the developing lung to cAMP.

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