Changes in Relative Levels of Guanosine-3':5'-Monophosphate-Dependent and Adenosine-3':5'-Monophosphate-Dependent Protein Kinases in Lung, Heart, and Brain of Developing Guinea Pigs  
(fetus/neonate/aging)

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ABSTRACT Changes in relative levels of protein kinases (ATP:protein phosphotransferase, EC 2.7.1.37) stimulated by either guanosine 3':5'-monophosphate (cyclic-GMP) or adenosine 3':5'-monophosphate (cyclic-AMP) were examined in extracts of the lung, heart, brain, and liver from guinea pigs at various stages of development. The level of cyclic-GMP-dependent protein kinase in the fetal lung, which was found to be the highest of any mammalian tissue samples examined, declined during development. On the other hand, the level of cyclic-AMP-dependent protein kinase in the same extracts, which was initially lower than that of the cyclic-GMP-dependent enzyme, increased during development and reached a level higher than that of the cyclic-GMP-dependent enzyme when the animals reached maturity. This reciprocal change in level of the two classes of protein kinases in developing lung was demonstrated further by chromatographing the extracts on Sephadex G-200 and quantitating the activity of the isolated enzymes. A decrease in the ratio of the two classes of protein kinases qualitatively similar to that seen in the lung was also noted in the developing heart. An increase in the ratio of the enzymes, however, was seen in the developing brain. Unlike in the lung, heart, and brain, no change in relative level and ratio of the enzymes was noted in liver during development.

These results suggest that a balance between the effects of cyclic-GMP-dependent and cyclic-AMP-dependent protein kinases may be important in normal development of certain tissues.

Recently it has been reported that cyclic-GMP-dependent protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37) occurs in high levels in mammals (1, 2), as shown earlier in the arthropods (3, 4). In view of current evidence that cyclic-GMP and cyclic-AMP may independently mediate opposing effects of certain physiological agents in the lung (5, 6) and other tissues and cells (see ref. 7 for a recent review), presumably via the phosphorylating activity of cyclic-GMP-dependent and cyclic-AMP-dependent protein kinases, respectively (3, 4, 8), it is conceivable that these two classes of protein kinases may be involved in many patho-physiological processes as well as in the normal development of tissues. The lung, which was shown to be particularly rich in the cyclic-GMP-dependent enzyme, would be an ideal tissue to study the development-related changes in the tissue levels of the cyclic-GMP target enzyme. In order to demonstrate any generalized pattern of changes, other representative tissues (such as the heart, brain, and liver) from guinea pigs at various stages of development were also examined. Levels of the cyclic-GMP-dependent and cyclic-AMP-dependent classes of protein kinases as well as their ratios were assessed. Changes in these parameters were indeed found to occur in certain developing tissues.

EXPERIMENTAL PROCEDURE

Materials. [γ-32P]ATP was purchased from New England Nuclear Corp.; cyclic-GMP and cyclic-AMP were from Boehringer Mannheim; arginine-rich histone (H3 or HA) was from Worthington Biochemical Corp.

Methods. Guinea pigs of both sexes and at different stages of development were used. They included the fetus (7–20 days before birth), the neonate (5 days postpartum), the young (42 days old), and the adult (310 days old). The lungs were dissected immediately after decapitation of the animals (including the fetus), rinsed in ice-cold 50 mM potassium phosphate buffer (pH 7.0), minced into small pieces, and homogenized in 3 volumes of the same buffer with glass-Teflon homogenizers. The homogenates were centrifuged for 20 min at 30,000 × g, and the supernatant solutions (crude extracts) thus obtained were used as the enzyme sources. Extracts from the heart, brain, and liver were similarly prepared.

In experiments in which separation and quantitation of cyclic-GMP-dependent and cyclic-AMP-dependent protein kinases present in lung extracts were involved, 6-ml aliquots (equivalent to 2 g of fresh tissue) of extracts were charged onto Sephadex G-200 columns (3.5 × 40.0 cm). The Sephadex was previously equilibrated with 50 mM phosphate buffer, pH 7.0, and the enzymes were washed from the columns using the same buffer, as described elsewhere (1).

The standard assay system (1) for protein kinase activity contained, in a final volume of 0.2 ml, potassium phosphate buffer, pH 7.0, 10 μmol; theophylline, 0.5 mmol; arginine-rich histone, 40 μg; MgCl2, 2 μmol; [γ-32P]ATP, 1 μmol, containing about 1.5 × 106 cpm; crude protein kinase modulator, 40–60 μg; with appropriate amounts of either cyclic-GMP or cyclic-AMP and enzyme preparations, as indicated. The reaction was carried out for 10 min at 30°C. One unit of enzyme activity is defined as that amount of enzyme that transferred 1 pmol of 32P from [γ-32P]ATP in recovered histone under the assay conditions.

Crude protein kinase modulator from rat liver was prepared through the steps of boiling of the extracts and precipitation of the factor from 5% trichloroacetic acid (9, 10).
The amount of protein was determined by the method of Lowry et al. (11). The statistical significance of the difference between two mean values was assessed by the Student's *t*-Test.

**RESULTS**

Protein kinase activity in extracts of guinea pig lungs at various stages of development, assayed in the presence and absence of cyclic-GMP or cyclic-AMP, was proportional to increasing amounts of enzyme protein added to the incubation system under the assay conditions (Fig. 1). The relative activity of the enzyme stimulated by the two cyclic nucleotides was markedly different in these groups of animals. Thus, the cyclic-GMP-stimulated protein kinase activity, compared to the cyclic-AMP-stimulated enzyme activity, was much higher in the fetus, only slightly higher in the neonate, and conversely lower in the adult (Fig. 1). A linear relationship between the enzyme activity and the incubation time (up to 15 min) was also noted for the same lung extracts used in experiments presented in Fig. 1 (data not shown).

Fig. 2 depicts further a reciprocal change in the relative activity of the enzymes stimulated by various concentrations of either cyclic nucleotide in extracts of developing lungs. The enzyme activity in the fetal lung was more sensitive to stimulation by cyclic-GMP than by cyclic-AMP, suggesting that it may contain a higher amount of cyclic-GMP-dependent protein kinase than that of the cyclic-AMP-dependent enzyme. Based upon the data shown (Fig. 2), the lung from the neonate seems to contain approximately equal amounts of the two classes of protein kinases, whereas the adult lung may contain a higher amount of the cyclic-AMP-dependent enzyme.

To define further the change in the relative enzyme activity stimulated by cyclic nucleotides seen in the crude extracts of developing lungs (Figs. 1 and 2), the levels of the cyclic-nucleotide-dependent protein kinases were analyzed in a more purified preparation. Aliquots (6 ml, equivalent to 2 g of fresh tissue) of the lung extracts pooled from the same groups of animals used for experiments in Figs. 1 and 2 were separately chromatographed on Sephadex G-200 columns (Fig. 3). As shown earlier (1), three protein kinase peaks were obtained for each lung sample; peaks 1 and 3 are cyclic-AMP-dependent, whereas peak 2 is cyclic-GMP-dependent. The size of both peaks of the cyclic-AMP-dependent enzyme progressively enlarged, whereas that of the cyclic-GMP-dependent enzyme peak conversely diminished during development. These results clearly demonstrate a reciprocal change in tissue level of the two cyclic-nucleotide-dependent protein kinases in developing lung, substantiating the similar observations made with crude extracts of the lung (Figs. 1 and 2).

Detailed studies examining the development-related changes in tissue levels of protein kinases and their ratio are illustrated in Fig. 4. The highest level of cyclic-GMP-dependent protein kinase and conversely the lowest level of the cyclic-AMP-dependent enzyme were found in the lung extracts from the fetus 20 days before birth, the earliest stage of development studied: As the lung developed, the cyclic-GMP-dependent enzyme level continued to decline, accom-

![Fig. 1](image1.png)

**Fig. 1.** Protein kinase activity in extracts of the lung from developing guinea pigs as a function of the amount of enzyme protein. Assay conditions were as described in the text except for the various amounts of enzymes used, as indicated. If present, the concentration of either cyclic nucleotide was 0.3 μM. The animals used were the fetus (7 days before birth), neonate (5 days postpartum), and adult (310 days old).

![Fig. 2](image2.png)

**Fig. 2.** Stimulation by various cyclic nucleotide concentrations of protein kinase in extracts of the lung from developing guinea pigs. Assay conditions were as described in the text except for the concentrations of cyclic nucleotides used as indicated. The amounts of the lung extracts used contained 12, 20, and 28 μg of protein for the fetus, neonate, and adult, respectively.

![Fig. 3](image3.png)

**Fig. 3.** Comparison of Sephadex G-200 chromatographic patterns of protein kinases in extracts of the lung from developing guinea pigs. The experimental procedure was as described in the text. In all cases, 6 ml of extracts obtained from 2 g of fresh lung tissue was used for the separation. The flow rate was 3 ml/10 min and the fraction size was 2.3 ml.
TABLE 1. Comparison of the estimated ratio of cyclic-GMP-dependent to cyclic-AMP-dependent protein kinase activity in extracts of the lung, heart, brain, and liver from developing guinea pigs

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Lung</th>
<th>Heart</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>2.14 ± 0.15*</td>
<td>0.32 ± 0.03†</td>
<td>0.04 ± 0.01†</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Neonate</td>
<td>1.55 ± 0.12‡</td>
<td>0.14 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Adult</td>
<td>0.45 ± 0.07</td>
<td>0.07 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>0.17 ± 0.03</td>
</tr>
</tbody>
</table>

Three to six guinea pigs from various developing stages (fetus, 10 days before birth; neonate, 5 days postpartum; adult, 310 days old) were used. The enzyme activity in each tissue extract was assayed in the presence and absence of 0.3 μM of either cyclic nucleotide as described in the text. The data presented are means (± standard errors) of the values obtained from each group of animals. The ratio of (G-PK/A-PK) is defined as in Fig. 4.

*Significantly different from the adult (P < 0.005).
†Significantly different from the fetus (P < 0.005).
‡Significantly different from the neonate (P < 0.01).

The age-related changes in the ratio of the two classes of protein kinases in extracts of other tissues from developing guinea pigs are compared in Table 1. As in the lung (also see Fig. 4), the ratio of cyclic-GMP-dependent to cyclic-AMP-dependent protein kinase level decreased in the heart during development. In the brain, however, the ratio increased during development. It is interesting that no change in the ratio of the two enzymes was noted in developing liver.

DISCUSSION

It has been shown that the lung is the richest source of cyclic-GMP-dependent protein kinase among all tissues of the adult guinea pig examined (1). The activity of the cyclic-GMP-dependent enzyme, however, is still only 40–60% of that of the cyclic-AMP-dependent enzyme activity in extracts of the lung from the adult animals (1). The present studies demonstrate that the fetal lung is a unique tissue in that it contains two to three times more cyclic-GMP-dependent enzyme activity than the cyclic-AMP-dependent enzyme activity. The lung of the guinea pig fetus, therefore, somewhat resembles the fat body of larvae and pupae of the silk-moth, which was shown earlier (4) to exclusively contain cyclic-GMP-dependent protein kinase activity; only a trace amount of cyclic-AMP-dependent enzyme activity could be detected.

The dynamic and reciprocal changes in the levels of cyclic-GMP-dependent and cyclic-AMP-dependent protein kinases in developing lungs are intriguing. An increase in cell number occurs in the actively growing and developing lung, as in the fetus. It has been hypothesized, based upon recent evidence (12–15), that cyclic-GMP may mediate the actions of mitogenic agents, whereas cyclic-AMP may inhibit cell proliferation. The present findings that the cyclic-GMP-dependent enzyme activity is higher than the cyclic-AMP-dependent enzyme activity in the fetal lung and that the relative level of the two classes of protein kinases changes in a reciprocal manner during development may be of some physiological significance. Several other cyclic nucleotide-related parameters in the developing lung and other tissues of guinea pigs should be explored. It would be interesting to see if a correlation exists between the contents of cyclic-GMP and cyclic-AMP, and the levels of their respective protein kinases in the developing tissues. The lung is a heterogeneous tissue consisting of many cell types. Increases in the kind as well as the number of cells occur during early stages of develop-
ment. Hence changes in level and ratio of cyclic-nucleotide-dependent protein kinases observed in the present studies may not necessarily reflect the development-related changes in a given cell type.

In the lung and heart, the ratio of cyclic-GMP-dependent to cyclic-AMP-dependent enzyme activity was higher in the fetus than in the neonate and adult, suggesting that the events mediated by cyclic-GMP-dependent protein kinase may be particularly important during the early stage of development of these tissues. Following the same line of reasoning, it seems that the events evoked by the same enzyme may be important, in contrast, at a late stage of development (i.e., aging) of the brain. Lack of a change in the ratio of the two classes of cyclic nucleotide target enzymes in a developing liver, on the other hand, suggests that events evoked by cyclic-GMP and cyclic-AMP probably remain relatively unchanged during the course of development of the animal.

Whether and how a balance between the effects of cyclic-GMP and cyclic-AMP, presumably mediated by the phosphorylating activity of the respective protein kinases, play a role in the developmental processes of these tissues are not yet known. Changes in levels and ratios of the two classes of protein kinases may also be involved in certain pathological conditions. We have recently observed that levels of cyclic-GMP-dependent protein kinase were much lower in the heart of spontaneously hypertensive rats (16), in the liver of genetically diabetic mice (16), and in the aorta of rabbits with experimental atherosclerosis (17). In all cases, the cyclic-AMP-dependent enzyme levels were only slightly affected or essentially remained unchanged.

Note Added in Proof. The developmental changes in cyclic-AMP-dependent protein kinase activity in extracts of guinea pig lungs assayed in the absence of protein kinase modulator were the same (18) as those assayed in the presence of the modulator as shown above. Since the modulator is required for the cyclic-GMP-dependent enzyme, changes in its activity could not be accurately measured in the absence of the modulator. Moreover, the developmental changes in the two classes of protein kinases were found to occur almost exclusively in the soluble (cytosol) fraction; only minimal changes in their relative activity were noted in the particulate fraction (18).

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