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Effects of dexamethasone on lung protein turnover

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Dexamethasone (2.5 mg/day per kg) treatment of young growing rats resulted in reduced food intake and rapidly inhibited whole-body and lung growth. Although the reduction in food intake partially explained the decrease in whole-body growth, it did not influence lung growth. After 24 h of dexamethasone treatment, ribosomal efficiency in the lung was reduced 44%, producing a 38% decrease in the rate of plumonary protein synthesis. Extending dexamethasone treatment to 5 days resulted in decreases in both ribosomal efficiency (35%) and capacity (28%), explaining the 53% reduction in lung protein synthesis at this time. After both the acute and chronic steroid regimes, the decreased rates of pulmonary protein synthesis were accompanied by a loss of polyribosomes and an elevated ribosomal monomer pool, indicating that dexamethasone blocked translation at the site of peptide-chain initiation.

INTRODUCTION

Growth retardation is a well known feature in children suffering from Cushing's syndrome and in those individuals receiving steroid therapy for prolonged periods (Blodgett *et al.*, 1956; Hyams & Carey, 1988). Animal studies have reinforced these clinical observations, demonstrating that glucocorticoid administration leads to a suppression of growth in young, and whole-body atrophy in adult, animals (Loeb, 1976; Tomas *et al.*, 1979; Kelly & Goldspink, 1982, 1983, 1984; Kelly *et al.*, 1986). More specifically, glucocorticoids exert a differential effect on protein metabolism in various tissues. Growth is enhanced in the liver (Munro, 1964) and heart (Kelly & Goldspink, 1982; Kelly *et al.*, 1986), reduced in slow-twitch skeletal muscle and abolished in fast-twitch skeletal muscle (Kelly & Goldspink, 1982; Kelly *et al.*, 1986). Lymphoid tissues show the greatest sensitivity to dexamethasone, with both the thymus and spleen undergoing marked atrophy (Kelly & Goldspink, 1983, 1984).

Glucocorticoids are increasingly being used in the treatment of acute lung injury (Schumer, 1976; Sibbald *et al.*, 1981) and recently for bronchopulmonary dysplasia (BPD), a chronic lung disease of premature infants (Mammel *et al.*, 1983, 1987; Cummings *et al.*, 1989). However, the growth response of the lung to these steroids has not been established. The present study was therefore undertaken to determine the sensitivity of the lung to the growth-suppressive effects of dexamethasone. The influence of acute (24 h) and chronic (5 days) administration of dexamethasone (2.5 mg/day per kg) on both growth and protein turnover in the lung of the adolescent rat was investigated. This protocol enabled a meaningful comparison of the study in the lung with earlier work in different tissues (Kelly & Goldspink, 1982, 1983, 1984; Kelly *et al.*, 1986). The changes in pulmonary protein metabolism induced by dexamethasone were further characterized by analysis of pulmonary ribosomal subunits and polysomal profiles.

EXPERIMENTAL

Animals

Male Wistar rats (36 in number, initial body wt. 100 g) were housed in a room controlled for temperature (22 °C) and having a daily photoperiod of 12 h light between 06:00 and 18:00 h. Animals were randomly divided into groups of six and housed in individual cages. Group 1 were killed immediately (i.e. day 0) and their lungs analysed as part of the growth-rate determinations. For the acute experiment, animals in groups 2 and 3 received a single subcutaneous injection of 0.9% NaCl or dexamethasone acetate (2.5 mg/kg suspended in 0.9% NaCl) respectively and were killed 24 h later. In the 'chronic' experiment, animals in groups 4 and 5 received a daily subcutaneous injection of 0.9% NaCl for 5 days, whereas group 6 received a daily subcutaneous injection of dexamethasone acetate (2.5 mg/day per kg) for 5 days. The food intake of groups 4 and 6 were recorded daily, and the average food intake of group 6 was given to group-5 animals (pair-fed controls) on the following day. Injections and body-weight recordings were undertaken between 09:00 and 10:00 h.

Determination of rates of protein synthesis

Protein synthesis was measured *in vivo* after an intravenous (i.v.) injection of phenylalanine to flood the precursor pool(s) (Garlick *et al.*, 1980). After the appropriate experimental treatment, rats from groups 2–6 were wrapped in a tea towel and injected with radiolabel via a lateral tail vein. The injection consisted of 150 μmol of phenylalanine, including 48 μCi of L-[4-^{3}H]phenylalanine (sp. radioactivity 28 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) in 1 ml of 0.9% NaCl/100 g body wt. At 10 min exactly animals were decapitated and a blood sample was collected in a heparinized tube over the following 15 s. The lung was then rapidly removed, weighed, frozen in liquid N_2 and stored at −20 °C until analysis. For animals in groups 4 and 6 a portion of unfrozen lung was retained for ribosomal profile analysis (see below). Sample preparation involved homogenizing the tissue (200 mg) in 5 vol. of ice-cold 0.2 M-$HClO_4$ with a ground-glass homogenizer. The specific radioactivities of phenylalanine, both free in the tissue pool(s) and covalently bound in protein, were measured in these homogenates as described by Garlick *et al.* (1980). This involved the prior hydrolysis of the washed protein pellets in 6 M-HCl at 110 °C for 12 h and the conversion of phenylalanine into β-phenylethylamine (Garlick *et al.*, 1980). Radioactivity measurements were made in an LKB 1219 liquid-scintillation counter after addition of Labscint scintillation fluid (Lablogic, Sheffield, U.K.), with the use of an external standard. The fractional rate

Abbreviation used: BPD, bronchopulmonary dysplasia.
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of synthesis (i.e. K_s, the percentage of the protein mass synthesized per day) was calculated from:

$$K_s = \frac{S_B}{S_A t} \times 100$$

where S_A and S_B are the specific radioactivities of phenylalanine in the free tissue pool (i.e. intracellular and extracellular) and protein respectively, and t is the time in days.

Daily growth rate (K_g) of the lung was calculated as the percentage change in protein mass occurring over the experimental period (i.e. between 0 and 1, or between 0 and 5 days), divided by the mean protein content (at 0.5 or 2.5 days). The total amount of protein synthesized in the lung was calculated as the product of the fractional synthetic rate and the protein content. Since the growth of a tissue arises as a consequence of imbalances between the rates of protein synthesis and rates of protein breakdown, the fractional rate of protein breakdown (K_b) was calculated from the predetermined growth rate (K_g) and fractional synthetic rate (K_s), i.e.:

$$K_b = K_s - K_g$$

Protein content was measured by the method of Smith *et al.* (1985), with BSA as a standard. RNA content was determined by alkaline hydrolysis as described by Fleck & Munro (1962). A value of $A_{260}^{32\ \mu g/ml} = 1$ was used to calculate the concentration of hydrolysed RNA.

Analysis of ribosomal particles and polyribosomes on sucrose density gradients

Lungs were minced with scissors and homogenized in ice-cold buffer containing 150 mM-KCl, 10 mM-Tris (pH 7.4, 20 °C) and 10 mM-$MgCl_2$ using 12 strokes of a dunce homogenizer (Rannels *et al.*, 1979). Homogenates were centrifuged at 10000 *g* for 10 min at 4 °C in the JA-20 rotor of a Beckman J2.2 refrigerated centrifuge. Triton X-100 was added to the resulting supernatants to give a final concentration of 1% (v/v). Detergent-treated supernatant samples (0.75 ml) were layered on to 0.58–1.46 M linear sucrose density gradients made up with homogenization buffer. Owing to the lower pulmonary RNA content after 5 days dexamethasone administration, the concentration of extract was adjusted to ensure an equivalent amount of RNA was loaded on to the sucrose density gradient. The gradients were centrifuged at 300000 *g* in a TST41.14 rotor (Kontron Instruments, Zürich, Switzerland) for 220 min or 7 h at 2 °C. The A_{254} of the gradients was monitored by using a UA-5 spectrophotomether (Instrumentation Specialities Co., Lincoln, NE, U.S.A., and fractions representing 40, 60, 80 S and polyribosomal particles were collected. RNA contents of homogenates and gradient fractions were determined by alkaline hydrolysis (Fleck & Munro, 1962).

Statistical analysis

The significance of difference between control and dexamethasone-treated groups was tested by the two-tailed unpaired Student's t test. Values of $P < 0.05$ were taken as being statistically significant.

RESULTS

Effects of dexamethasone on food intake, whole-body and lung growth

Our previous studies with dexamethasone administration at 2.5 mg/day per kg have shown an immediate effect on whole-body and tissue growth in the rapidly growing adolescent rat. However, it had not been established whether these changes were due in part to reduced food intake in the steroid-treated animals.

In a preliminary study, dexamethasone administration (2.5 mg/day per kg) was found to reduce food intake by the second day from 17.0 to 9.5 g/day per animal. Food intake remained at this level for the next 3 days. Whole-body and lung growth were both suspended in these animals (results not shown). In order to determine the relative contributions of steroid treatment and reduced food intake on lung growth, a second series of animals, incorporating pair-fed controls, were studied.

Rats injected daily with saline and fed *ad libitum* grew at an average rate of 6.0%/day (Fig. 1). By contrast, after 48 h of dexamethasone treatment, animals were losing weight at an average rate of 3.0% per day. At this time, food intake had fallen by 40% and remained at this level over the following 3 days (results not shown). Pair-fed saline-injected controls gained weight at 7.0%/day over the first 2 days; thereafter body weight remained constant (Fig. 1). Lung growth, measured as protein accumulation, was abolished within 24 h of dexamethasone administration and remained suppressed over 5 days of steroid treatment. In contrast with whole-body growth, lung growth of pair-fed control rats did not differ from *ad libitum* controls, indicating that lung growth was unaffected by this degree of food restriction (Table 1).

Effect of dexamethasone on pulmonary protein turnover

The rapid cessation of lung growth in rats treated acutely with dexamethasone was due to a large fall (38%) in the rate of pulmonary protein synthesis (Table 2). After this marked initial reduction, the rate of lung protein synthesis decreased even further during the chronic phase of steroid treatment. The high rate (42.1 ± 3.4%) of lung protein synthesis of pair-fed saline-control rats further verified that reduced food intake after dexamethasone administration did not contribute to the steroid-induced changes in pulmonary protein turnover.

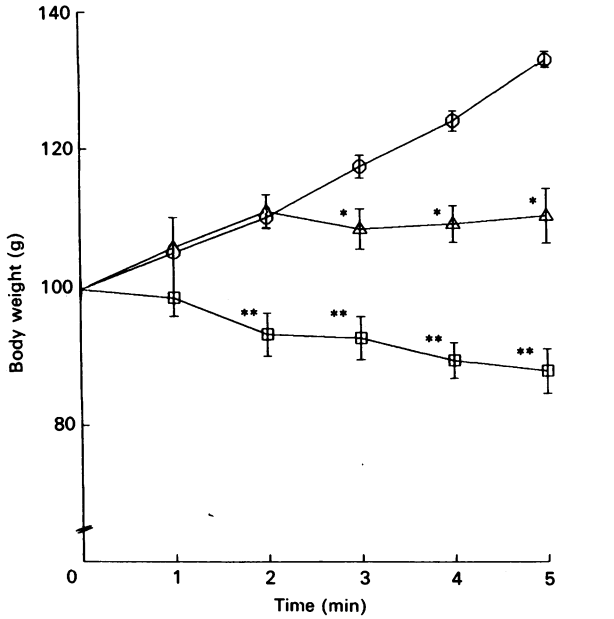

Fig. 1. Changes in whole-body growth in response to dexamethasone or pair-feeding with animals receiving dexamethasone

Rats initially weighing 100 g were given daily subcutaneous injections of 0.9% NaCl (○) or 2.5 mg of dexamethasone/kg body wt. (□). A third group of animals (△) received saline and were pair-fed with those rats receiving dexamethasone. Body weights were measured between 09:00 and 10:00 h each morning over the experimental period. Each point represents the mean ± S.E.M. for six animals. Significance: *$P < 0.01$, pair-fed versus '*ad libitum*' controls; **$P < 0.001$, dexamethasone versus '*ad libitum*'- and pair-fed controls.

Table 1. Effect of dexamethasone on the rate of lung growth

Total protein (collagenous and non-collagenous) was measured by the method of Smith *et al.* (1985). Growth rates were derived from changes in the protein mass between days 0 and 1 and between days 0 and 5. The values presented are means ± S.E.M. derived from six animals. Values in parentheses show the percentage change from the day 5 '*ad libitum*'-fed control group. Statistical significance for the dexamethasone group versus both control groups on day 5: $*P < 0.01$.

Duration of treatment (days)	Treatment	Protein content (mg)	Daily growth rate (%/day)
0		86.8 ± 7.1	
1	Control	94.3 ± 4.8	8.3
	Dexamethasone	85.2 ± 3.6	−0.7
5	Control	113.3 ± 4.6	5.2
	Pair-fed control	114.4 ± 4.9	5.3
	Dexamethasone	85.3 ± 4.9* (−25%)	−0.3

The decrease in the rate of protein synthesis in the lung 24 h after dexamethasone exposure was partially compensated for by a small decrease (17%) in the calculated rate of pulmonary protein breakdown. After 5 days of steroid administration, protein breakdown in the lung was further depressed (46%), in parallel with the greater fall in protein synthesis at this time point (Table 2).

The mode of action of dexamethasone on pulmonary protein synthesis was investigated by calculating RNA/protein values and synthesis per unit of RNA in the lung. These represent indices of ribosomal capacity and efficiency respectively (Henshaw *et al.*, 1971; Waterlow *et al.*, 1978). During the acute phase of steroid administration, the reduced rate of protein synthesis in the lung was due to a significant fall in translational efficiency (44%), whereas synthetic capacity was unaltered (Table 2). Upon extending dexamethasone treatment to 5 days, significant losses in both capacity (35%) and efficiency (28%) explained the low rate of pulmonary protein synthesis.

Effects of dexamethasone on the ribosomal cycle of the lung

Sucrose-gradient analysis of polyribosomal and ribosomal aggregation in the lung after dexamethasone treatment was used to further define the reduced efficiency of translation. Although dexamethasone administration did not change the relative proportion of 40 S and 60 S subunits compared with controls, an increased content of 80 S monomers was evident in the lung after both acute (Fig. 2*a*) and chronic (Fig. 2*c*) steroid treatments. A 25–30% increase in the RNA content of the peak representing the 80 S monomeric ribosome in the lung after dexamethasone supports this observation (results not shown). The loss of polysomal material in the lungs of steroid-treated rats (Figs. 2*b* and 2*d*), accompanied by a elevated monomer population, indicates that a block at peptide-chain initiation is responsible for the steroid-induced inhibition of pulmonary protein synthesis.

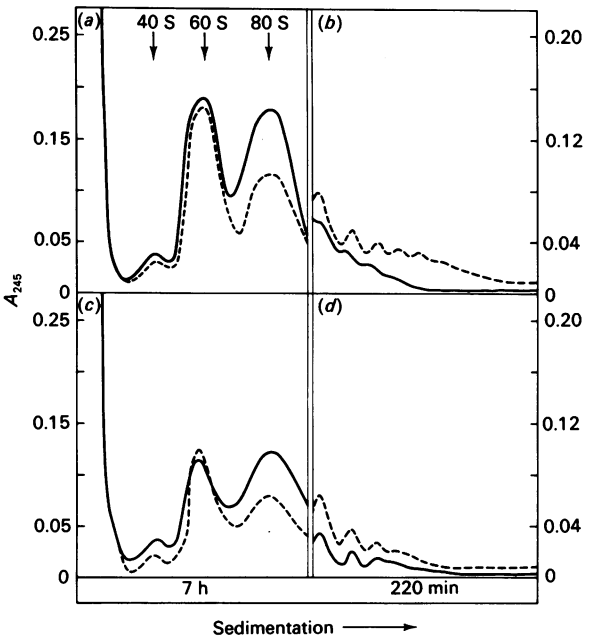

Fig. 2. Sucrose-density-gradient analysis of polyribosomes and ribosomal particles from lungs of rats treated with dexamethasone

Lungs from rats treated for 24 h (*a* and *b*) or 5 days (*c* and *d*) with either saline (----) or dexamethasone (——) were homogenized as described in the Experimental section. The postmitochondrial supernatant was layered on to linear 0.59–1.46 M sucrose gradients. Ribosomal subunits and monomers (*a* and *c*) and polysomes (*b* and *d*) were resolved by centrifugation at 300000 *g* in a TST41.14 rotor (Kontron Instruments) for 7 h and 220 min respectively. Fractions were monitored for absorbance at 254 nm.

DISCUSSION

Glucocorticoids are frequently used in the treatment of both neonatal and adult lung disease. In particular, broncho-

Table 2. Effects of dexamethasone on protein turnover of the lung

The fractional synthesis rate of protein was determined as described by Garlick *et al.* (1980). The fractional breakdown rate was calculated from the fractional rate of synthesis and the growth rate ($K_b = K_s - K_g$). The efficiency of protein synthesis represents the total protein synthesized per mg of RNA. The values presented are means ± S.E.M. and are derived from six animals. Values in parentheses show the percentage change from the respective control groups. Statistical significance for the dexamethasone versus the control groups: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Duration of treatment (days)	Treatment	Fractional rate of synthesis (%/day)	Fractional rate of breakdown (%/day)	Ribosomal efficiency (total protein synthesized/mg of RNA)	Ribosomal capacity (RNA/protein)
1	Control	37.4 ± 2.1	29.1	20.9 ± 2.4	20.2 ± 1.2
	Dexamethasone	23.4 ± 2.2* (−38%)	24.1 (−17%)	11.7 ± 1.5* (−44%)	19.9 ± 1.7
5	Control	41.6 ± 3.4	36.4	17.8 ± 1.6	23.5 ± 0.9
	Dexamethasone	19.4 ± 0.9*** (−53%)	19.7 (−46%)	11.7 ± 0.5** (−35%)	16.9 ± 0.8*** (−28%)

pulmonary dysplasia (BPD), a chronic lung disease of preterm infants, appears to be particularly responsive to short-term steroid therapy (Mammel *et al.*, 1983, 1987; Cummings *et al.*, 1989). Successful growth, maturation and repair of lung tissue in premature infants with BPD is of utmost importance if they are to break free from dependence on respiratory support. Well-established side-effects associated with dexamethasone therapy, such as growth suppression, would suggest caution in prolonging or increasing the dose of steroid in the treatment of BPD.

In the present study the protein mass, and hence growth, of the lung was clearly sensitive and highly responsive to elevated circulating glucocorticoid concentrations (Table 1). The rapid and complete cessation of lung growth induced by dexamethasone was due mainly to the steroid's effect on protein synthesis. The rate of pulmonary protein synthesis decreased markedly within 24 h of steroid treatment and fell further after 5 days of dexamethasone administration (Table 2). Similar rapid responses to steroid treatment have also been observed in lymphoid tissue (Kelly & Goldspink, 1983, 1984). The extent to which the rate of protein synthesis was reduced in the lung was of similar magnitude to that reported in the tibialis anterior, 37% (Kelly & Goldspink, 1982), extensor digitorum longus, 31% and the plantaris, 25% (Kelly *et al.*, 1986) muscles which undergo a marked atrophy in response to dexamethasone. The ability of the lung to resist a steroid-induced catabolism was due to the compensatory fall in the rate of protein breakdown (Table 2).

A reduction in the rate of protein synthesis may result either from a decrease in the amount of tissue RNA, hence a compromised capacity to synthesize protein (Millward *et al.*, 1973), or from the inefficient use of the synthetic capacity available or by a combination of these two mechanisms. By investigating the effects of acute (24 h) and chronic (5 day) dexamethasone administration, its primary and secondary actions on pulmonary protein metabolism were elucidated. The initial fall in the rate of lung protein synthesis induced by dexamethasone was due solely to a reduction in translational efficiency. The additional fall in protein synthesis occurring over 5 days of steroid treatment was due to combined reductions in ribosomal capacity and efficiency. Sequential changes in ribosomal efficiency, followed by capacity, have previously been reported in skeletal muscle in response to insulin deficiency (Pain *et al.*, 1983) and in the liver after nutrient deprivation (Millward *et al.*, 1973). In addition, changes in both ribosomal capacity and efficiency have been observed in smooth and skeletal muscle after 5 days of exposure to glucocorticoids (Rannels *et al.*, 1978; Rannels & Jefferson, 1980; Kelly & Goldspink, 1982; Kelly *et al.*, 1986).

The compromised efficiency to synthesize protein in the lung was further investigated by defining the step in translation impaired by dexamethasone. Elevated populations of 80 S monomers were found in the lungs of both acutely and chronically steroid-treated rats (Figs. 2*a* and 2*c*). As the dissociation of monomeric ribosomes is a prerequisite for initiation of protein synthesis (Sabol & Ochoa, 1971), this indicated that protein synthesis was limited by the reactions of peptide-chain initiation. This conclusion was further confirmed by polyribosome disaggregation, which accompanied the increased 80 S monomer content in the lung after glucocorticoid treatment (Figs. 2*b* and 2*d*). An impairment in peptide-chain initiation after glucocorticoid treatment has previously been reported in skeletal muscle 4 h after dexamethasone administration or 5 days after cortisone acetate treatment (Rannels *et al.*, 1978; Rannels & Jefferson, 1980). Peptide-chain initiation involves a complex series of events (reviewed by Pain, 1986). Although the exact point of initiation which is regulated by dexamethasone has not been clarified in the present work, it may be considered in the context of what has been observed in other tissues. For example, changes in Met-tRNA^{fMet} binding in skeletal muscle in response to insulin deficiency (Harmon *et al.*, 1984; Kelly & Jefferson, 1985) and glucocorticoid excess (Rannels *et al.*, 1978) suggest that the specific process affected is the formation of 40 S initiation complexes mediated via a change in initiation-factor activity.

The present study has clearly shown that lung growth is particularly sensitive to dexamethasone. The dose of dexamethasone employed, namely 2.5 mg/day per kg, is comparable with that used clinically in severe cases of BPD. The subject material, adolescent rats, although not ideally representative of preterm infants, did provide important information regarding the effects of corticosteroid therapy on lung growth. Rats, unlike humans, are born in a very immature state, and considerable lung growth and development occurs after birth (Burri *et al.*, 1974). For instance, lung growth in the adolescent rats used in this study was still 5%/day. Using animals at this more manageable size, we found that lung growth was particularly sensitive to the growth supressive effects of dexamethasone.

These data, in conjunction with other work indicating that lung growth is compromised in the presence of the high concentrations of O_2 required by many infants (Kelly, 1988), would suggest that caution is required in the application of dexamethasone therapy to treat BPD.

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