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Rat lung lectin synthesis, degradation and activation

Developmental regulation and modulation by dexamethasone

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Soluble lectins are widely distributed cell-agglutinating proteins. Their activity is developmentally regulated in several tissues, including the lung, but virtually nothing is known about the mechanisms of the developmental regulation or the turnover of these proteins. We studied mechanisms that might be responsible for the developmentally regulated changes in the activity of a lectin (β-galactoside-binding protein) found in the lung, and determined if its activity or turnover could be modulated by treatment of rat pups with a glucocorticosteroid hormone (dexamethasone). Our studies on the activity and turnover of the lectin indicated that the peak of lectin activity (units/mg of protein) that occurred at age 12 days appeared to be brought about by two means: an increase in the activity of the lectin molecule itself (units/μg of lectin) that occurred at age 8 days, and 1.5-fold increase in the absolute rate of lectin synthesis at age 11 days. The decline in lectin activity was associated with a decrease in its rate of synthesis, return to the baseline extent of activation, and an increased rate of degradation. Treatment of rat pups with dexamethasone diminished the peak of lectin activity (units/mg of protein) by about 25%. This effect of dexamethasone was due, at least in part, to the complete prevention of activation of the lectin molecule (units/μg of lectin) and a premature increase in the rate of lectin degradation. Perhaps the normal fall in lectin activity after age 11 days is caused by mechanisms induced by the increase in serum corticosteroid that occurs at that age.

INTRODUCTION

Lectins are sugar-specific cell-agglutinating proteins found in plants, micro-organisms and animals (Barondes, 1981). Those in vertebrates may be divided into two classes: integral membrane lectins, which require detergents for their extraction, and soluble lectins (Barondes, 1981, 1984). Most of the soluble vertebrate lectins are β-galactoside-specific, and they have been found in organs of teleosts, amphibia, birds and mammals. The best-studied avian and mammalian lectins are dimers, with subunit M_r values near 14000; thiol reducing agents, but not Ca^{2+} or other cations, are required for lectin activity. The physiological function of the soluble lectins is unknown, but the activity of these lectins is developmentally regulated in liver, kidney, muscle, intestine and lung (Beyer & Barondes, 1982; Powell & Whitney, 1980). The mechanism by which lectin activity reaches a maximum at a particular stage of development and then declines has not been previously reported, nor is there any information about agents that modulate lectin activity.

The lectin that is the subject of this paper is the dimeric β-galactoside-binding protein of subunit M_r 14000. In the lungs of all species tested (guinea pig, hamster, rat) the activity of this lectin rises to a peak during the time when the lung's gas-exchange saccules are being converted into mature alveoli: this occurs *in utero* in the guinea pig (Maxwell *et al.*, 1984), a few days after birth in hamsters (Maxwell *et al.*, 1984), and in the second postnatal week in the rat (Powell & Whitney, 1980). It is of some interest that in guinea pigs and rats (information not available in hamsters) a substantial rise in the serum concentration of the species' active glucocorticoid occurs at about the time of peak lectin activity (Dalle & Delost, 1976; Henning, 1978), suggesting that these hormones might serve to regulate lectin activity.

We now show in rat lungs that the developmentally regulated peak of lectin activity is associated with an increased rate of lectin synthesis and an activation of the lectin molecule (increased activity/μg of lectin). We also show that activation of the lectin and its turnover can be hormonally modulated; activation is completely prevented by treatment of rat pups with a glucocorticosteroid (dexamethasone), whereas its synthesis and degradation are increased.

MATERIALS AND METHODS

Materials

Animals and breeding. Adult Sprague–Dawley albino rats were obtained from Charles River Laboratories, Portage, ME, U.S.A. They were allowed food (Rodent Laboratory Chow 5001; Ralston–Purina Co., St. Louis, MO, U.S.A.) and water *ad libitum*. Lighting was provided from 07:00 to 19:00 h. Breeding was accomp-

Abbreviations used: KRB, Krebs–Ringer bicarbonate; TCA, trichloroacetic acid; Cam-lectin, carboxamidomethyl-lectin; PAGE, polyacrylamide-gel electrophoresis.

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lished by placing two females with one male overnight. Probable pregnancy was determined the next morning by the presence of sperm in a smear made from vaginal contents. Pups were designated to be 1 day old the day after birth; they were raised ten per litter, and the litters were adjusted to that size within 18 h of birth. Rats were killed between 09:00 and 11:00 h by exsanguination after anaesthesia with pentobarbital sodium (approx. 60 mg/kg, intraperitoneally).

Lung slices. After death, the lungs were removed from the thorax, rinsed externally in cold Krebs–Ringer bicarbonate (KRB) medium (118 mM-NaCl/4.7 mM-KCl/2.5 mM-$CaCl_2$/1.2 mM-KH_2PO_4/1.2 mM-$MgSO_4$/25 mM-$NaHCO_3$, pH 7.4) and sliced with a McIlwain tissue chopper (Brinkman Instruments, Westbury, NY, U.S.A.) set to make 1.0 mm slices. In all instances the medium was equilibrated with CO_2/air (1:19) before use, and all incubations were carried out in a gas phase of CO_2/air (1:19). Unless stated otherwise incubations were carried out in medium containing 5.5 mM-glucose, 0.7 mM-phenylalanine and adult rat serum concentrations of the other 19 amino acids (Morgan *et al.*, 1971).

Precursor pools specific radioactivity

We determined the specific radioactivity of intracellular and medium phenylalanine as previously described in detail (Thet *et al.*, 1977). Phenol extraction, as reported by Airhart *et al.* (1979), was used to isolate aminoacyl-tRNA, and the tRNA was deacylated by alkali treatment. Samples of extracellular and intracellular fluids and of amino acids obtained by deacylation of tRNAs were placed on Dowex 50W-X4 (200–400 mesh) cation-exchange resin, eluted with aq. 5.0 M-NH_3, and dried under a stream of nitrogen. The dried samples were dissolved in 0.6 ml of sodium citrate buffer (pH 2.2), and analysed in a JEOL amino acid analyser by using split-stream fraction collection to allow measurement of the amount and radioactivity of phenylalanine. A known amount of norleucine was added to all starting materials to permit us to account for phenylalanine lost during the extraction procedures.

Protein synthesis

Lung slices were incubated for 1 h at 37 °C. At the end of the incubation, the medium was replaced by fresh medium, identical with the preincubation medium except that it contained 0.7 mM-[^{14}C]- or -[^{3}H]-phenylalanine instead of 0.7 mM non-radioactive phenylalanine. The slices were re-incubated for 1–4 h at 37 °C, the medium was removed, TCA added to a final concentration of 10% (w/v), and the mixture centrifuged at 400 *g* for 10 min. A sample of the supernatant fluid was assayed for its radioactivity and phenylalanine content, and these data were used to calculate the specific radioactivity of extracellular phenylalanine (Thet *et al.*, 1977).

To measure radioactivity incorporated into total protein and to determine the specific radioactivity of intracellular phenylalanine, lung slices were removed from the incubation flasks, and rinsed with ice-cold normal saline (0.15 M-NaCl) until the washes were free of radioactivity. Lung slices were then disrupted in 10% TCA at 0–4 °C with a Polytron operated at full speed for 1 min with a PT 10 ST probe. The homogenized tissue was kept on ice for 20 min and then centrifuged at 400 *g* for 10 min. The supernatant fluid was decanted, and its radioactivity and phenylalanine content were measured, and these values were used in calculating the specific radioactivity of intracellular phenylalanine (Thet *et al.*, 1977). The TCA-insoluble pellet was washed three times with ice-cold 10% TCA and extracted with 5% TCA at 90 °C for 15 min. After centrifugation at 400 *g* for 10 min, the supernatant fluid was removed and its DNA content was assayed; the protein precipitate was dissolved in 0.2 M-NaOH and its radioactivity was measured.

During the course of our experiments we learned that the concentrations of amino acids in serum of newborn rats are considerably higher than those in the serum of adult rats (Girard *et al.*, 1975). To determine whether the rate of protein synthesis by lung slices from early postnatal rats was influenced by the use of adult concentrations of amino acids in the medium, we measured protein synthesis at adult or neonatal concentrations of amino acids. In lung slices from day-1 rats the rates of synthesis (±S.E.M.) were 27.3±0.6 and 26.3±0.8 nmol of phenylalanine incorporated/h per mg of DNA with adult (Morgan *et al.*, 1971) and neonatal (Girard *et al.*, 1975) amino acid concentrations respectively. In lung slices from adult rats the rates of synthesis were 20.9±1.1 and 19.3±0.8 respectively. Since the amino acid composition of the medium did not alter the rate of protein synthesis by lung slices from neonatal or adult rats, incubations were carried out in adult-rat serum concentrations of amino acids.

Lectin synthesis

To measure lectin synthesis, lung slices were incubated without radioactive phenylalanine and then incubated with [^{3}H]phenylalanine as described above. At the end of the incubation, the slices were washed in ice-cold 0.15 M-NaCl, frozen in liquid nitrogen, and subsequently homogenized in a Polytron in 20 mM-sodium phosphate buffer (pH 7.2)/140 mM-NaCl/10 mM-dithioerythritol/10 mM-lactose. [^{14}C]Cam-lectin, which was prepared as described by Whitney *et al.* (1986), was added to the homogenate to allow us to estimate the lectin lost during the isolation procedure. The disrupted tissue was centrifuged at 27000 *g* for 1 h at 4 °C, and the supernatant material was dialysed against 20 mM-sodium phosphate buffer (pH 7.2)/140 mM-NaCl/25 mM-mercaptoethanol to remove lactose. The material remaining after dialysis was chromatographed on a column (1.8 cm × 0.5 cm) of lactosyl-Sepharose CL-6B prepared as described by Levi & Teichberg (1981). The lectin was eluted with buffer containing 10 mM-lactose and was subjected to SDS/PAGE. The gel was sliced and radioactivity extracted by incubation of the slices in 0.3 ml of Soluene-350 for 16 h at 56 °C. During electrophoresis [^{14}C]Cam-lectin, our internal standard, co-migrated with the major radioactive peak of material eluted from the lactosyl–Sepharose column.

Protein and lectin degradation

To label lung proteins with [^{3}H]phenylalanine, we incubated lung slices for 1 h at 37 °C in KRB medium containing adult-rat serum concentrations of 19 amino acids, 5.5 mM-D-glucose, and trace amounts of [^{3}H]phenylalanine (200 μCi of [*side-chain*-^{3}H]phenylalanine; 25 Ci/mmol). After 1 h, the slices were removed, washed thoroughly in cold KRB and re-incubated for 8 h with KRB medium containing adult-rat serum concentra-

tions of 19 amino acids, 5.5 mM-D-glucose and 10 mM-L-phenylalanine. This high concentration of L-phenylalanine was used to prevent the re-incorporation into protein of [^{3}H]phenylalanine released by the degradation of radioactive proteins (see below). At various times incubations were stopped by chilling the flasks; the lung slices were immediately removed, washed with ice-cold saline, and frozen in liquid nitrogen. Protein and lectin were isolated from the slices as described above in the procedure to study their synthesis. Radioactivity in protein and in lectin was measured and expressed per mg of DNA. In separate experiments we determined if the presence of 10 mM-L-phenylalanine in the medium would alter the rate of protein degradation. To do this, we preincubated lung slices for 1 h with trace amounts of [^{3}H]leucine. The lung slices were then divided into two groups and placed in identical fresh medium without radioactive leucine; one group of slices was incubated with 0.7 mM-L-phenylalanine, and one with 10 mM-L-phenylalanine. After the incubation, the procedure was the same as described above.

Phenylalanine content of lung protein

We hydrolysed TCA-insoluble material from lung tissue in 6 M-HCl at 10 °C for 20 h. Samples of the supernatant material were analysed for phenylalanine on a JEOL amino acid analyser by single-column methodology.

Lectin purification and assay

Lectin was purified by affinity chromatography and gel filtration and was assayed by measuring agglutination of trypsin-treated rabbit erythrocytes in Cooke micro-titre plates as previously described (Whitney *et al.*, 1985). One unit of lectin activity is defined as the ability to agglutinate red cells at 1000-fold dilution of lectin. Activity was expressed as units/mg of protein or units/μg of lectin.

Preparation of anti-lectin antiserum

New Zealand White female rabbits (4–6 weeks old) were injected subcutaneously in the dorsal region at multiple sites with an emulsion containing equal volumes of Cam-lectin (600 μg) and Freund's complete adjuvant. This treatment was followed, at 2-week intervals, by subcutaneous booster injections with an emulsion containing equal volumes of Cam-lectin (300 μg) and Freund's incomplete adjuvant.

Electroblotting

Our procedure was described in the operating instructions of the Trans-Blot Cell from Bio-Rad. After SDS/PAGE, protein in the gel was electrophoretically transferred to nitrocellulose paper. This paper was then coated with 3% (w/v) gelatine, incubated with rabbit anti-lectin antibody and then with Protein A–colloidal gold and developed with hydroquinone/silver lactate, fixed and dried (Danscher & Norgaard, 1983).

Lectin immunoassay

Inhibition enzyme-linked immunosorbant assay was used to quantify lectin. Cam-lectin (5 μg/ml) was immobilized to wells of polyvinyl micro-titre plates; the plates were then coated with 1% bovine serum albumin in phosphate-buffered saline (123 mM-NaCl/10.4 mM-Na_2HPO_4/3.2 mM-KH_2PO_4, pH 7.4) to prevent non-specific adsorption of subsequently added reagents. Antiserum (diluted 1:8000) was preincubated for 18 h at 4 °C alone, with known concentrations of lectin, or with samples whose concentration of lectin was being determined. After incubation, samples of these mixtures were added to triplicate wells of the prepared micro-titre plates and incubated at room temperature for 45 min. The wells were then washed with phosphate-buffered saline containing 0.05% Tween 20. Goat anti-rabbit immunoglobulins linked to alkaline phosphatase (Zymed, San Francisco, CA, U.S.A.) were added to the wells; the plates were incubated for 90 min and again washed. *p*-Nitrophenyl phosphate, the substrate for the alkaline phosphatase, was added. The enzyme reaction was stopped after 1 h by the addition of 2 M-NaOH, and the A_{405} was read in a Titertek multiscan instrument (Flow Laboratories). The results were expressed as percentages of the A_{405} values in wells on the same micro-titre plate in which only phosphate-buffered saline was used for the preincubation step. Lectin in the range of 0.1–1800 ng/well was used to construct a standard curve. Lectin concentrations in tests were 1–50 ng/well.

Chemical determinations and radioactivity measurement

Protein was measured by the Bradford (1976) method, with bovine serum albumin as the standard. DNA was extracted and measured by the method of Schneider (1957), with calf thymus DNA (Sigma Chemical Co., St. Louis, MO, U.S.A.) as a standard. Samples to be assayed for radioactivity were placed in scintillation fluid [Aquasol for aqueous samples, or 5 g of 2,5-diphenyloxazone and 0.26 g of 1,4-bis-5-(phenyloxazol-2-yl)-benzene per litre of toluene for gel slices in Soluene] and counted in a Packard Prias scintillation counter. We corrected for quenching with an external standard.

Hormones

Dexamethasone was purchased as Hexadrol phosphate (4 mg/ml) from Organon, West Orange, NJ, U.S.A. Starting on postnatal day 4, rats were injected subcutaneously with 0.25 μg of dexamethasone (0.01 mg/ml in 0.075 M-NaCl)/day; other rats received an equal volume of diluent (0.075 M-NaCl). This dosage was chosen on the basis of previous studies on the effects of dexamethasone on lung development (Massaro *et al.*, 1985).

Statistical analysis and calculations

For each parameter calculated from measurements, the values for individual animals were averaged per experimental group and the S.E. of the group mean was calculated. The significance of the difference between two groups was obtained by using a paired or unpaired *t*-test analysis (Snedecor & Cochran, 1967). The significance of the difference between more than two groups was obtained by Duncan's (1955) multiple-range test. Calculations of intracellular, extracellular and tRNA-bound specific radioactivity were done as previously described (Thet *et al.*, 1977; Airhart *et al.*, 1979).

RESULTS AND DISCUSSION

General protein synthesis

We chose phenylalanine as a marker to measure protein turnover because it is an essential amino acid for rats (Rose *et al.*, 1948), and neither rat lung slices (Thet

Table 1. Ratio of precursor-pool specific radioactivities in the presence of 0.7 mM-phenylalanine

Lung slices were incubated with 0.7 mM-phenylalanine (45 Ci/mol) for 15–60 min. We then determined the specific radioactivity of phenylalanine released from tRNA, and the specific radioactivities of intracellular (IC) and extracellular (EC, medium) phenylalanine. Mean values ± S.E.M. are given, for the numbers of experiments in parentheses.

	Incubation period		
	15 min	60 min	
	tRNA/EC	tRNA/EC	IC/EC
2 days	0.96±0.03 (2)	1.00±0.06 (3)	0.98±0.02 (7)
Adult	0.99±0.01 (2)	0.95±0.02 (6)	0.97±0.02 (15)

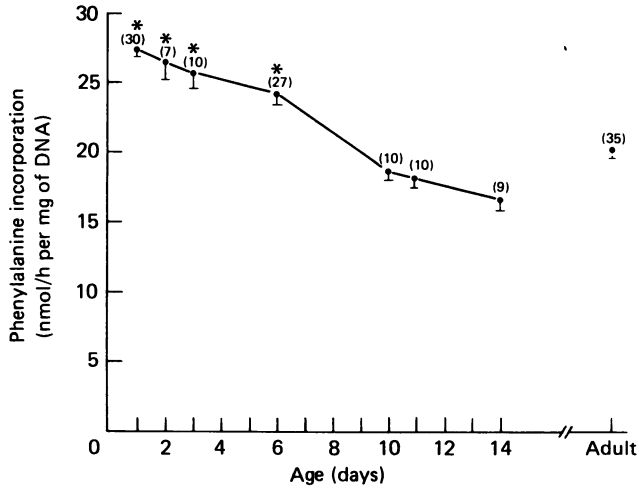

Fig. 1. Absolute rates of general protein synthesis in lung slices

Means ± S.E.M. are shown. The numbers in parentheses represent the numbers of experiments at each age. *$P < 0.05$ for each day (1, 2, 3 and 6) compared with each older day studied.

et al., 1977) nor the intact rat lung (Chiang *et al.*, 1979; Watkins & Rannels, 1980) convert phenylalanine into other compounds. The lung of adult rats contains 0.32 nmol of phenylalanine/μg of protein (Watkins & Rannels, 1980). Since we planned to study the incorporation of phenylalanine into lung proteins by neonatal as well as adult rats, we measured the phenylalanine content of lung protein (nmol/μg of protein) at both ages; we found 0.32±0.01 (mean±S.E.M., $n = 5$), 0.31±0.01 ($n = 3$) and 0.32±0.02 ($n = 3$) in lungs from 5-day-old, 10-day-old and adult rats respectively. Thus the phenylalanine concentration in lung proteins was unchanged over much of the age range during which we measured protein synthesis and degradation.

In the intact perfused and ventilated adult rat lung, the specific radioactivity of intracellular and perfusate phenylalanine becomes equal to that of tRNA-bound phenylalanine by 1 h, when the concentration of phenylalanine in the perfusate is 0.7 mM (Watkins & Rannels, 1980). We have now shown that with 0.7 mM-phenylalanine in the medium a similar equilibration was reached within 15 min in slices of neonatal and adult lungs (Table 1). This has allowed us to use the specific radioactivity of phenylalanine in the medium to calculate the absolute rate of general protein and lectin synthesis by lung slices. The rate of general protein synthesis fell steadily during the first 2 postnatal weeks, and was significantly higher at days 1, 2, 3 and 6 than on each older day studied (Fig. 1). The fractional rates of synthesis, calculated from the rates of synthesis, also fell from ages 1 to 14 days (results not shown).

The rate of protein synthesis that we found in slices from adult rat lungs is virtually identical with that reported in the intact perfused adult rat lung (Watkins & Rannels, 1980); the fractional rate of protein synthesis in our adult rat lung slices (16.5%/day) is close to that found *in vivo* (20%) (Kelley *et al.*, 1984). The rate of protein synthesis at day 6 (24.2 nmol incorporated/h per mg of DNA) is approx. 80% of that achieved by the lung in experiments *in vivo* on 6-day-old pups (M. Hass & D. Massaro, unpublished work). These observations lead us to conclude that the rates of protein synthesis in slices of adult and neonatal rat lungs are a reasonable reflection of the rate of lung protein synthesis *in vivo*.

General protein degradation

For the purpose of these studies, the method chosen to measure general protein degradation also had to be applicable to measure lectin degradation. We first labelled the lung slices by preincubation for 1 h with [^{3}H]phenylalanine and then followed the loss of labelled protein or lectin during incubation of the slices in unlabelled medium. Although this procedure gives an accurate measure of lectin degradation, the short labelling period preferentially labels lung proteins that turn over rapidly. For instance, in studies with perfused adult rat lung, the rate of protein degradation was 11%/h for lung prelabelled for 10 min, compared with 3%/h for lung prelabelled for 5 h (Chiang *et al.*, 1979). These considerations indicate that our measurements of general protein degradation are dominated by the proteins that turn over rapidly. Since protein synthesis does measure total synthesis, our results cannot be used to assess the balance of protein synthesis and degradation. Previous consideration of this balance in adult rat lung slices led to the conclusion that there was a positive protein balance, in agreement with expectations of continued lung growth *in vivo* (Massaro, 1985). Therefore lung slices appear to be a reasonable system for studies of protein turnover.

When radiolabelled amino acids are used to study the rate of protein degradation, the re-incorporation into protein of marker amino acids released from protein by proteolysis can lead to an underestimation of the rate of proteolysis. We found that with 2 mM-phenylalanine in the medium only 0.02% of [^{3}H]phenylalanine derived from protein degradation was re-incorporated into protein. Thus the high concentration of phenylalanine in the medium almost completely prevented the re-incorporation into protein of phenylalanine made available for protein synthesis by degradation of lung protein. We next sought some assurance that the high concentration of phenylalanine used for this purpose did not alter protein degradation. We therefore incubated lung slices with radioactive leucine for 1 h, after which

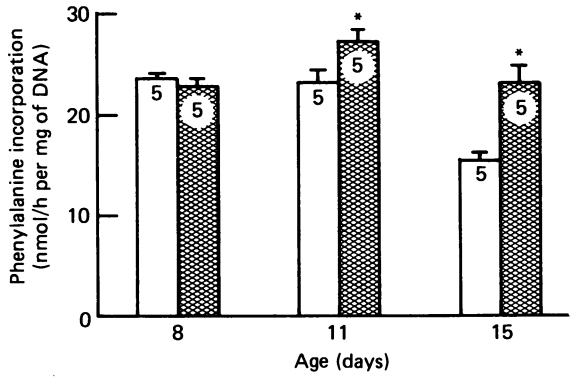

Fig. 2. Effect of dexamethasone treatment on protein synthesis in lung slices

Postnatal rats were treated daily with 0.075 M-NaCl diluent (□) or 0.25 μg of dexamethasone/day (▩), starting on day 4, and studied at ages 8, 11 and 15 days. Means ± S.E.M. are shown. The numbers inside the bars indicate the numbers of experiments. *$P < 0.05$ compared with diluent-treated rats of the same age.

Table 2. General protein degradation

Rat pups were treated daily with diluent (0.075 M-NaCl) or dexamethasone (0.25 μg/day), starting on day 4. General protein degradation (%/h) was measured as described in the Materials and methods section. Values are means ± S.E.M. for three experiments.

Age (days)	Treatment...	Degradation (%/h) Uninjected	Diluent	Dexamethasone
4		4.6±0.5		
9			5.2±0.3	5.2±1.0
11			4.3±0.3	4.3±0.3
13			4.2±0.4*	6.7±0.1***
15			7.3±0.4**	9.3±0.8**

* $P < 0.05$ versus day-13 dexamethasone-treated rats.
** $P < 0.05$ versus preceding values in the same column and day-4 uninjected rats.
*** $P < 0.05$ versus day-11 dexamethasone-treated rats.

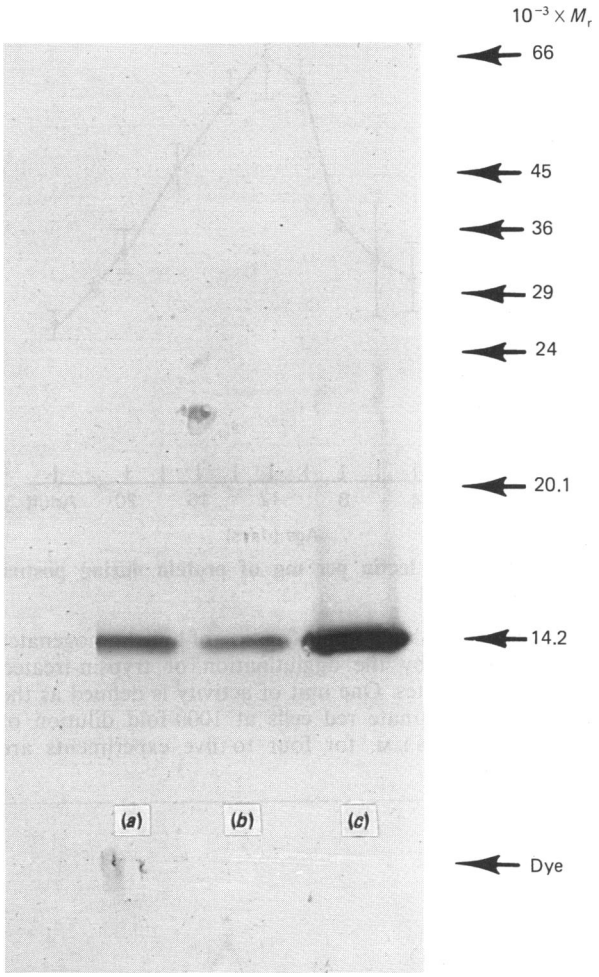

Fig. 3. Electrophoretic immunoblot from SDS/PAGE-treated lung homogenate

Lanes (*a*) and (*b*) represent 27000 *g*-supernatant material of lung homogenates from two different 11-day-old rats; lane (*c*), pure lectin (approx. 1 μg). The numbers on the right indicate the migration of M_r standards. Staining was done with Protein A–gold, followed by silver lactate.

the slices were washed and incubated without radioactive leucine but with 0.7 mM- or 10 mM-phenylalanine. In two experiments, degradation of proteins from slices incubated with 0.7 mM-L-phenylalanine was 1.7%/h and 1.4%/h. Degradation of proteins from slices incubated with 10 mM-L-phenylalanine was 1.6%/h and 1.2%/h. These values indicate that the presence of 10 mM-L-phenylalanine did not change the overall rate of protein degradation.

Dexamethasone treatment and the turnover of general proteins

The rate of general protein synthesis was not different in lung slices from diluent- and dexamethasone-treated rats at age 8 days (4 days after the onset of treatment) (Fig. 2). At ages 11 and 15 days the rates of general protein synthesis were higher in lung slices from dexamethasone-treated pups than from diluent-treated rats. The difference between groups was greatest at age 15 days; this was brought about by a 35% decrease of synthesis from the rate at day 11 in rats injected with diluent, compared with a 14% decrease in those injected with dexamethasone.

General protein degradation in lung slices from diluent-treated rats was significantly higher at day 15 than at the earlier ages tested (Table 2). In lung slices from dexamethasone-treated rats, general protein degradation was increased by day 13. In a comparison between diluent- and dexamethasone-treated rats at each day, the rate of degradation was significantly different on day-13 lungs ($P < 0.05$).

Anti-lectin antibody specificity

We subjected 27000 *g*-supernatant fluid from lung homogenates of 11-day-old rats to SDS/PAGE. Proteins in these gels were electroblotted on to nitrocellulose and then allowed to react with rabbit anti-lectin antiserum. We also subjected pure lectin (subunit M_r 14000) to

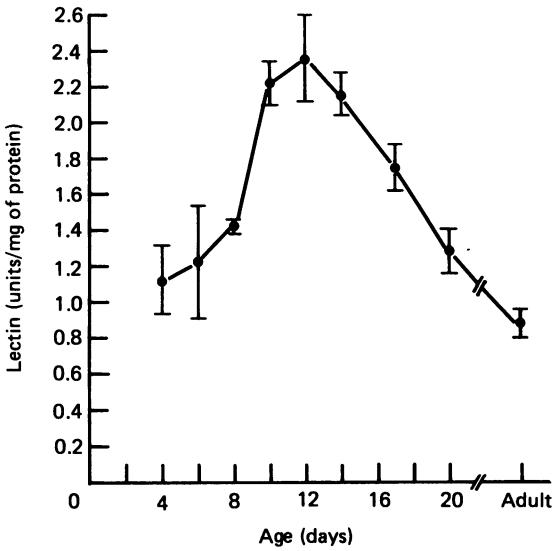

Fig. 4. Activity of lectin per mg of protein during postnatal development

Lectin activity in the soluble fraction of lung homogenates was measured by the agglutination of trypsin-treated rabbit erythrocytes. One unit of activity is defined as the ability to agglutinate red cells at 1000-fold dilution of lectin. Means ± S.E.M. for four to five experiments are shown.

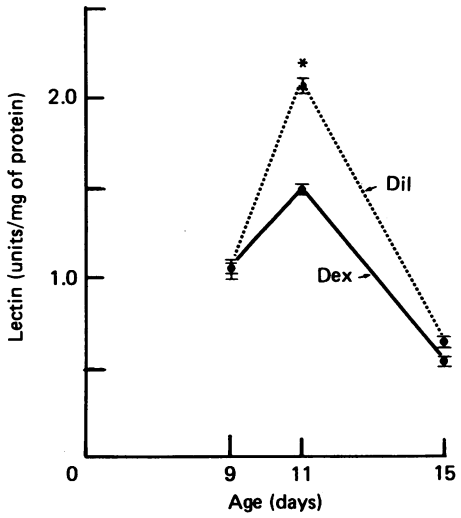

Fig. 5. Effect of dexamethasone treatment on lectin activity (units/mg of protein)

Rats were treated daily with diluent (Dil) or 0.25 μg of dexamethasone/day (Dex), starting on day 4. Means ± S.E.M. for 19 experiments are shown: $^*P < 0.05$ compared with day-11 Dex-treated pups.

similar treatment. The antiserum reacted with pure lectin (Fig. 3, lane *c*). The antiserum also reacted with material in lung homogenates that had the same electrophoretic mobility as pure lectin (Fig. 3, lanes *a* and *b*). We did not observe staining when pre-immune serum was used in place of anti-lectin serum. We concluded that the specificity of the antiserum allowed its use to quantify lectin in homogenates of postnatal lungs.

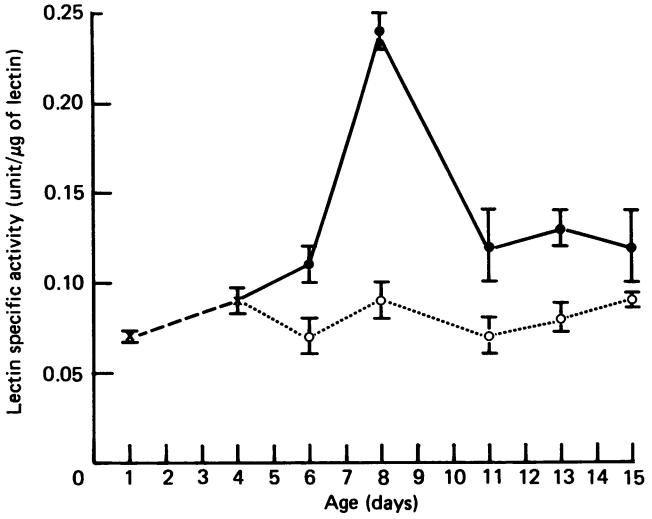

Fig. 6. Specific activity of lectin (units/μg of lectin)

We measured lectin specific activity in homogenates from uninjected (×) rat pups at ages 1 and 4 days and in homogenates from rat pups treated with diluent (●) or 0.25 μg of dexamethasone/day (○), starting on day 4. Activity was measured by agglutination of trypsin-treated rabbit erythrocytes, and lectin was quantified by inhibition enzyme-linked immunosorbant assay. Means ± S.E.M. for three to five experiments are shown. The activity of diluent-treated rats at day 8 was significantly higher than for dexamethasone-treated rats ($P < 0.001$).

Lectin activity

The activity of the lectin per mg of soluble protein increased from day 4 to day 12, and then decreased (Fig. 4). This time course is similar to that previously reported from this laboratory (Powell & Whitney, 1980), but the present peak is lower, and we cannot explain the difference. Treatment with dexamethasone from age 4 days had no effect on the lectin activity (units/mg of protein) up to age 9 days (results not shown). By age 11 days, the lectin activity of dialysed homogenate supernatant material from lungs of dexamethasone-treated rats was 72% that of diluent-treated rats, but by age 15 days the values of diluent- and dexamethasone-treated rats were not different (Fig. 5).

The time course of the haemagglutinating activity per molecule of lectin (units/μg of lectin) differed somewhat from the lectin activity in the homogenate (units/mg of protein). The haemagglutinating activity per molecule was about 2.7-fold higher on postnatal day 8 than on previous days, and then decreased to values that were slightly greater than those present before postnatal day 8 (Fig. 6). The peak of activity per molecule of lectin did not occur in lungs of dexamethasone-treated pups.

The data in Fig. 6 are calculated from the results of two assays that tend to have relatively low precision. However, the peak of specific activity noted in lungs from diluent-treated animals at age 8 days represents results from five separate experiments done with different animals. Statistically the difference between diluent- and dexamethasone-treated groups at age 8 days is highly significant ($P < 0.001$).

These differences in specific activity could be due to differences in reactivity in the immunoassay, to altered

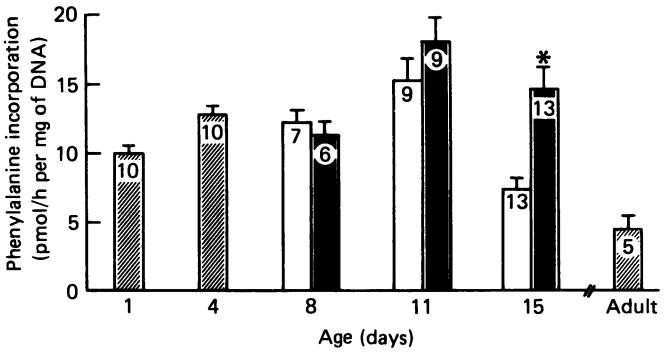

Fig. 7. Rates of lectin synthesis in lung slices from postnatal rats

We measured the incorporation of [^{3}H]phenylalanine into lectin that was isolated by affinity chromatography on lactosyl–Sepharose followed by SDS/PAGE. Recovery of [^{14}C]Cam-lectin was used to account for losses during purification. Experiments were done on uninjected rat pups at postnatal days 1 and 4 (■) and on rat pups that were treated daily either with diluent (□) or 0.25 μg of dexamethasone/day (■), starting on day 4. Rates of synthesis were expressed as pmol of phenylalanine incorporated into lectin/h per mg of DNA. Means ± S.E.M. are shown. The numbers in the bars represent the numbers of experiments performed. *$P < 0.05$ compared to age-15-days diluent-treated rats. In lung slices from diluent-treated rats, the rates of lectin synthesis were higher at day 11 than at day 8 ($P < 0.05$), and both day-8 and -11 synthesis rates were greater than for day 15 ($P < 0.05$). With dexamethasone treatment, day-11 synthesis was higher than on day 8 ($P < 0.05$), but was not different from that on day 15.

Table 3. Lectin degradation

Rat pups were treated daily with diluent (0.075 M-NaCl) or dexamethasone (0.25 μg/day), starting on day 4. Lectin degradation (%/h) was measured as described in the Materials and methods section. Values are means ± S.E.M. for three experiments.

Age (days)	Treatment...	Degradation (%/h) Uninjected	Diluent	Dexamethasone
4		4.8 ± 1.2		
9			4.5 ± 0.3	4.4 ± 0.5
11			4.7 ± 0.1	3.9 ± 0.4
13			4.6 ± 0.3*	9.2 ± 1.4***
15			11.1 ± 2.0**	7.3 ± 0.4***

* $P < 0.05$ versus day-15 dexamethasone-treated rats.
** $P < 0.01$ versus preceding values in the same column and day-4 uninjected rats.
*** $P < 0.05$ versus day-9 and -11 dexamethasone-treated rats and day-4 uninjected rats.

amounts of an inhibitor of lectin activity, or to molecular changes in the lectin that would give higher activity. To test the last possibility, we measured the specific activity of purified lung lectin from diluent- and dexamethasone-treated rat pups (age 8 days). In three experiments, the specific activity of lung lectin from diluent-treated rats was 42 ± 9% (mean ± S.E.M.) higher than that for lectin from dexamethasone-treated rats. The biochemical basis for these differences in specific activity has not yet been identified.

Lectin turnover

The time course of the rate of lectin synthesis (Fig. 7) paralleled that of the lectin activity (Fig. 4). The increases do not reflect the rate of general protein synthesis, since the latter fell over this period (Fig. 1). On postnatal day 15 the rate of lectin synthesis was greater in lung slices from dexamethasone-treated rats than in slices from diluent-treated rats. This action of dexamethasone on lectin synthesis was similar to the effect of dexamethasone on general protein synthesis (Fig. 2).

The rise in specific activity of lectin from age 4 to 11 days was not brought about by a decreased rate of lectin degradation (Table 3). The rate of lectin degradation was virtually the same in diluent-treated pups at age 4, 9, 11 and 13 days, but was 2.5-fold higher on postnatal day 15 (Table 3). The findings were similar in lungs from dexamethasone-treated pups, but the increase in the rate of degradation occurred by postnatal day 13. In a comparison between diluent- and dexamethasone-treated rats on each day, the rate of degradation was significantly greater in day-13 lung slices from dexamethasone-treated rats ($P < 0.05$).

Mechanisms of developmental control

Our studies indicate that the developmentally regulated increase in lectin activity per mg of protein, which reaches a peak at about postnatal day 11, is brought about by the combination of an increased absolute rate of lectin synthesis and an increase in haemagglutinating activity per lectin molecule. Changes in the rate of lectin degradation do not seem to play a role in the increased activity of the lectin per mg of protein. The fall in lectin activity per mg of protein seems to be brought about by a fall in the rate of lectin synthesis, diminished activation, and an increased rate of lectin degradation. The time course of the rate of lectin synthesis does not, but the rate of lectin degradation does, mirror the rate of general protein synthesis and degradation.

It is possible that the change in the rate of lectin synthesis is due to a quantitative alteration in the lung's population of fibroblasts or endothelial cells, cells previously shown to synthesize lectin (Whitney *et al.*, 1985). Endothelial cells do not increase and then decrease in number during this time, but there is evidence that a class of lung fibroblasts, the so-called 'lipid-ladened fibroblasts', change in a manner that somewhat parallels the change in lectin synthesis and activity (Massaro & Massaro, 1986). Thus, if the peak of lectin activity is due to a change in the cellular composition of the lung, the fibroblast is likely to be most responsible for the change.

The three processes that affect lectin activity per mg of lung protein (haemagglutinating activity per molecule, lectin synthesis and lectin degradation) are all influenced by dexamethasone treatment. This observation and the time course of the changes in these processes and in the serum concentration of corticosterone, which rises at approximate age 12 days (Henning, 1978), suggest that adrenal corticosteroids may have a physiological role in the developmentally regulated changes of lectin activity in the lung.

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