Dexamethasone increases plasma amino acid concentrations in bronchopulmonary dysplasia

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Abstract

Nine ventilated low birthweight babies were treated with dexamethasone (0.6 mg/kg/day). Appreciable suppression of weight gain was accompanied by uraemia and significant increases in the concentration of all amino acids except phenylalanine, tyrosine, threonine, and glutamate. Ornithine, citrulline, alanine, glutamine, and cystine concentrations increased threefold or more. The findings could not be explained by changes in dietary intake and presumably reflect pronounced catabolism, though the effects of dexamethasone on intermediary metabolism and membrane transport could also play a part.

Dexamethasone is used increasingly to treat infants with bronchopulmonary dysplasia, principally to facilitate the withdrawal of mechanical respiratory support.^{1,2} The precise mode of action of this drug in bronchopulmonary dysplasia is not clear, but lung compliance is known to increase, possibly through clearance of interstitial oedema. Treatment, which may be given for six weeks or more,² is usually accompanied by suppression of weight gain and uraemia, indicating the presence of catabolism.³ The metabolic vulnerability of immature infants provided with protein in excess of growth requirements is well known,⁴⁻⁷ but there are no data about the metabolic effects of high dose corticosteroid treatment in this group of patients. In this study we have measured the effect of dexamethasone on plasma amino acid concentrations.

Patients and methods

PATIENTS

The infants studied had been mechanically ventilated from birth. Table 1 gives the birth weight and gestational and postnatal age of each. Dexamethasone was used in the presence of radiographic abnormalities suggestive of chronic lung disease when conventional respirator weaning techniques had failed. The decision to treat was taken by the clinician responsible and no other experimental intervention to the standard nursery protocol was made. The dose of dexamethasone employed was 0.6 mg/kg/day in three divided doses given intravenously or orally.

FEEDING

The method of feeding was determined by each infant's clinical state and not regulated for the

purpose of this study. None of the infants was completely enterally fed and all were receiving commercial parenteral nutrition solutions (Vamin Infant, Kabi Pharmacia, 10% dextrose $\pm$ 20% Intralipid, Kabi Pharmacia), as a part or the whole of their nutrient intake. The parenteral nutrition regimens used in our nursery provide between 167 and 502 kJ/kg/day, depending on lipid tolerance, and amino acid intake is adjusted to constrain protein equivalent: energy ratio between 3.35 and 6.70 g/MJ. Enterally fed infants received low birthweight formula (Cow and Gate) or human milk when available. Total nitrogen and energy intakes were calculated from nursing records and manufacturers' compositional data. The nitrogen content of human milk was assumed to be 200 mg/100 ml, and the energy content 251 kJ/100 ml or 293 kJ/100 ml, depending on whether banked milk (a mixture of dripped and expressed milk) or mother's own milk respectively was used.

ANTHROPOMETRY

Weight (g) was measured daily when clinical state permitted, using an averaging electronic scale with resolution of 1 g and precision of $\pm$ 4 g. Weight gain was calculated as the linear regression coefficient (slope) of these measurements on time (days). This was divided by body weight at the commencement of treatment for comparative expression in units of g/kg/day. Although such ratio 'correction' can lead to erroneous conclusions when comparison is made between infants of differing size, we made comparison only within subjects between treatment periods.

BIOCHEMICAL ANALYSIS

Heparinised venous blood samples (0.5 ml) were drawn before the first dose of dexamethasone and on one occasion between two and seven days later when a clinical indication

Table 1 Clinical characteristics of the infants studied

Patient No	Birth weight (g)	Gestation (weeks)	Postnatal age (days)
1	1041	29	16
2	664	24	32
3	2300	32	13
4	842	28	18
5	726	24	22
6	830	25	45
7	1048	26	31
8	918	26	17
9	715	27	22
Median	842	26	22

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for routine sampling arose. Plasma was separated immediately and frozen to -20°C . A 200 μl aliquot was deproteinised with 20 μl of 35% (w/v) sulphosalicylic acid after the addition of 200 μl of 250 $\mu\text{mol/l}$ L-norleucine, which was used as an internal standard. The mixture was vortexed for a few seconds before incubating at room temperature for 5 minutes and then centrifuging again for 5 minutes at 15 850 g. An aliquot of 350 μl of the deproteinised supernatant was mixed with an equal volume of Beckman lithium diluent (ph 2.2) and the sample either stored at -60°C or filtered through a 0.22 μm filter for immediate analysis.

Amino acid concentrations were measured using a cation exchange high performance liquid chromatography (HPLC) system (Beckman System Gold) with postcolumn ninhydrin derivatisation, detecting primary amino acids and related compounds at 570 nm wavelength. The system was calibrated using a 250 $\mu\text{mol/l}$ Beckman physiological standard. For each infant, specimens collected before and during treatment were analysed sequentially to ensure compatible measurement conditions. The precision (coefficient of variation) of the assay procedure was established by replicate assay ($n=8$) of the same sample of adult blood. It lay between 1.5 and 5.2% for all amino acids except citrulline (10.7%).

Plasma urea was determined by the urease method on a random access analyser in the hospital routine laboratory.

STATISTICAL ANALYSIS

The number of patients studied was small ($n=9$) and most variables did not appear normally distributed. Consequently the Wilcoxon signed rank test has been employed to make comparisons before and during treatment within infants. Two tailed probability estimates (p) are quoted throughout; values of <0.05 were deemed significant.

Results

NITROGEN AND ENERGY INTAKE DURING STUDY

Table 2 shows the average daily nitrogen and energy intake of each baby during the seven days before and the seven days after dexamethasone was commenced. These, and protein:energy ratio, did not differ significantly between the two periods (Wilcoxon signed rank test). There was a small difference in the ratio of

Figure 1 Weight gain (g/kg/day) during the week before commencing dexamethasone and during the first week of treatment.

enteral to parenteral intake between treatment periods: the median proportion of total fluid intake as milk before treatment was 26% (range 0–91%) and 19% (range 0–66%) during treatment. This difference was not significant (Wilcoxon signed rank test, $p>0.1$) and was attributable to interruption of enteral feeding on endotracheal extubation.

WEIGHT GAIN

Figure 1 shows tied values for average daily weight gain or loss during the week before and the week after commencing dexamethasone treatment. In one case insufficient weight data were obtained during the second week to calculate rate of change. In six of the remaining eight cases there was a net weight loss on treatment. These changes were significant (Wilcoxon signed rank test, $p<0.05$).

PLASMA UREA CONCENTRATIONS

Figure 2 shows the distribution of plasma urea values with time. There was a clear increase in

Table 2 Nitrogen, energy intakes, and weight velocity of infants studied

Patient No	Before dexamethasone			After dexamethasone		
	Nitrogen intake (mg/kg/day)	Energy intake (kJ/kg/day)	Weight velocity (g/kg/day)	Nitrogen intake (mg/kg/day)	Energy intake (kJ/kg/day)	Weight velocity (g/kg/day)
1	246	368	-5.8	324	431	-5.2
2	350	439	6.9	36	401	-11.7
3	107	205	20.0	98	134	*
4	326	477	41.3	236	397	-21.2
5	356	418	26.0	259	380	-5.3
6	392	435	25.4	363	380	-12.3
7	293	330	8.8	449	531	-2.8
8	287	318	15.7	410	414	-19.1
9	289	405	7.7	330	410	13.4
Median	293	405	15.7	330	401	-8.5

*Denotes insufficient data to calculate velocity.

Figure 2 Plasma urea values (mmol/l) during the week before commencing dexamethasone and during the first week of treatment.

both the median plasma urea concentration and variability between individuals during treatment.

PLASMA AMINO ACID CONCENTRATIONS

Table 3 shows the median concentration (with range) for each amino acid before and after treatment. Significant increases were observed in all except glutamic acid, threonine, phenylalanine, and tyrosine concentrations (Wilcoxon signed rank test; *p* values given in table 3). The uniformity of the changes across infants was striking; in the case of most of the amino acids an increase was observed in every infant studied,

notable exceptions being threonine, glutamic acid, phenylalanine, and tyrosine. The most appreciable increases were noted in ornithine, citrulline, cystine, methionine, glutamine, and alanine concentrations (fig 3).

Discussion

Dexamethasone treatment was associated with suppression of weight gain and striking increases in the plasma concentrations of most amino acids and urea. These changes could not be attributed to differences in nitrogen and energy intake between treatment periods. Our assumptions about the composition of human milk are unlikely to have influenced comparative nutrient intakes. This is firstly because the quantities employed were small and, secondly, because only within subject, between period comparisons were made. The small, statistically non-significant changes in the disposition of nutrient intake between parenteral and enteral routes are also unlikely to have been important, particularly as the protein:energy ratio was constrained during parenteral nutrition. Moreover, the pattern of hyperaminoacidaemia we describe differs from that previously observed by Rih *et al* in dietary protein excess.⁴⁻⁷ Concentrations of alanine, glycine, ornithine, lysine, histidine, serine, and threonine measured during dexamethasone treatment in our study were greater than those observed by Rih *et al* in low birthweight infants fed unadjusted cows' milk protein at 4.5 g/kg/day. Conversely, the concentrations of leucine, isoleucine, valine, phenylalanine, tyrosine, citrulline, glutamate, and

Figure 3 (A-D) Plasma amino acid concentrations in mol/l. Values for samples taken before and after commencing dexamethasone are tied for individual infants. Dotted line indicates 90th centile value for ad libitum term breast fed infants.¹⁸

Table 3 Concentrations of plasma amino acids before and after treatment

	Median concentration ($\mu\text{mol/l}$) before treatment (range)	Median concentration ($\mu\text{mol/l}$) after treatment (range)	p Value	Median concentration ($\mu\text{mol/l}$) for breast fed term infants ¹⁸ (10th, 90th centile)
Ornithine	45 (24–171)	207 (195–287)	<0.001	84 (49, 116)
Citrulline	11 (8–24)	34 (16–58)	<0.001	13 (6, 20)
Cystine	11 (3–28)	34 (16–79)	<0.001	52 (34, 62)
Taurine	48 (22–236)	89 (46–256)	<0.001	50 (27, 96)
Methionine	29 (12–39)	55 (30–94)	<0.001	20 (13, 29)
Alanine	161 (83–362)	572 (371–992)	<0.001	235 (163, 387)
Asparagine	35 (8–96)	87 (58–120)	<0.001	53 (43, 87)
Glutamine	228 (114–440)	651 (258–902)	<0.001	610 (468, 755)
Glutamic acid	76 (64–341)	96 (68–368)	NS	56 (36, 98)
Glycine	295 (123–509)	558 (388–1007)	<0.001	183 (120, 228)
Serine	191 (86–316)	415 (213–599)	<0.001	109 (80, 143)
Threonine	176 (60–430)	260 (188–491)	NS	104 (76, 134)
Lysine	127 (43–278)	280 (194–410)	<0.001	148 (92, 210)
Histidine	74 (39–218)	136 (114–260)	<0.001	66 (44, 85)
Leucine	94 (67–164)	154 (103–170)	<0.002	103 (73, 160)
Valine	176 (120–274)	265 (143–391)	<0.001	153 (108, 184)
Isoleucine	40 (14–80)	59 (32–99)	<0.05	49 (32, 71)
Proline	150 (84–586)	281 (170–708)	<0.001	212 (162, 283)
Phenylalanine	75 (53–230)	94 (66–239)	NS	41 (23, 63)
Tyrosine	115 (62–1548)	130 (44–853)	NS	72 (45, 117)

cystine which we measured were lower, and those of methionine, proline, glutamine, and taurine similar to those described by Rih *et al.* The low cystine concentration measured in our study is particularly noteworthy and probably reflects the relatively high requirement for parenteral nutrition and low intake of human milk.

It seems unlikely that the plasma amino acid concentrations attained were intrinsically harmful as none approached levels characteristic of metabolic disease. Indeed, neonatal tyrosinaemia observed in one infant of 24 weeks' gestation improved after 48 hours of dexamethasone treatment, perhaps as a result of tyrosine aminotransferase induction.⁸ The complexity of the metabolic response to steroid treatment makes it difficult, however, to be certain of the mechanisms underlying the changes that we have observed. The most important is likely to be a change in the balance between rate of protein synthesis and breakdown, but corticosteroid induced changes in active transport of amino acids across membranes could also affect cellular uptake. For example, corticosteroids reduce amino acid uptake by skeletal muscle and lymphoid tissue but increase uptake by hepatocytes.⁹ The effects of corticosteroids on intracellular metabolism appear similarly complex—for example, hydrocortisone treatment both increased carbamoyl phosphate synthetase-I activity and decreased ornithine transcarbamoylase activity in fetal rat liver.¹⁰ It is not clear to what extent the changes we observed in the urea cycle intermediates ornithine and citrulline were due to an overall increase in protein oxidation or to what extent any inhibition of ornithine transcarbamoylase might have been responsible.

Although the changes induced by glucocorticoids are often described as 'catabolic', protein turnover studies have suggested that nitrogen wasting results both from a decrease in protein synthesis and a rise in degradation rates,^{11 12} though the latter may fall in some tissues.¹² Skeletal muscle has been considered to show the most appreciable effects. In the rat, those muscles with the highest proportion of fast fibres (for example, extensor digitorum longus)

show the most noticeable atrophy and reduction in protein synthesis, whereas those with a greater proportion of slow fibres (for example, soleus) tend to be spared. The diaphragm, which contains a mixture of fast and slow fibres, appears to behave in an intermediate manner.^{12 13} At present, there are no data on the effect of corticosteroids on diaphragmatic structure and function in the immature human infant.

The effect of corticosteroids on skeletal muscle has also been extensively studied in mature dogs. Muhlbacher *et al* showed a significant efflux of glutamine from skeletal muscle in animals given 0.44 mg/kg/day of dexamethasone.¹⁴ However, the changes which they observed in plasma amino acid concentrations differ from those which we have noted in immature infants. Most notably, ornithine, glutamine, and cystine concentrations fell while alanine increased by a factor of only 1.5. In contrast, rats treated with 6 mg/kg/day of dexamethasone showed a rise in plasma glutamine concentration accompanied by efflux of glutamine and alanine from the lung.¹⁵ This is of interest because the human and the rat, unlike the dog and other species studied, appear to release glutamine from the lung.¹⁵ A recent observation that dexamethasone treatment appreciably suppresses lung growth and protein synthesis in the adolescent rat¹⁶ raises the possibility that the increased plasma alanine and glutamine concentrations we describe here could be attributable as much to suppression of protein synthesis in the lung as in skeletal muscle. By analogy with the effects of dietary protein restriction in the rat this might be expected to increase lung compliance¹⁷ and produce short term clinical improvement, but also to slow the rate of repair.

In summary, we observed striking increases in the plasma concentrations of most amino acids in immature infants treated with dexamethasone. These changes may reflect suppression of protein synthesis in both the lung and the respiratory musculature, but the relative contributions of effects of membrane transport and intermediary metabolism remain to be determined.

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