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Glucocorticoid receptors in circulating lymphocytes of premature infants and newborns

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The number and affinity of glucocorticoid receptors in lymphocytes of newborns and prematures were determined by a whole cell ^{3}H-dexamethasone binding assay. Mean receptor numbers were, 1758 ± 245/cell in cord blood, 2758 ± 307/cell in mature newborns and 2025 ± 485/cell in prematures. Three of the premature babies died with hyaline membrane disease (HMD). They had not been treated prenatally with dexamethasone and no specific binding was measurable in their lymphocytes, suggesting that the lack of receptors might be one of the causes of HMD

Recently it has been established that several mammalian tissues display a significant number of glucocorticoid receptors [1, 3, 5, 12, 20]. Receptors have been analysed also in a number of diseases [5, 9] and correlations have been found in several cases between receptor number and the clinical outcome [11].

Hyaline membrane disease (HMD) is a major cause of neonatal mortality, especially in premature infants. Glucocorticoids are known to accelerate maturation of the lungs and they have been administered as prenatal therapy in recent years. Since the introduction of this therapy, the frequency and mortality of HMD have significantly been reduced [18].

Our aim was to compare the number of glucocorticoid receptors in peripheral lymphocytes of healthy term newborns, prematures, and of babies suffering from HMD. It was hoped that based on this kind of data, it will be possible to tell in retrospect why prenatal glucocorticoid therapy for the prevention of HMD was successful in some cases, but not in others.

MATERIALS AND METHODS

Patients

The patients were 20 mature newborns and 20 prematures of 32.7 ± 2.17 weeks gestational age. Blood samples were taken from a peripheral vein, sometimes through the umbilical catheter 48–72 h after birth, at 8 o'clock a.m. Clotting was inhibited by heparin. Umbilical blood samples were taken also from ten normal babies delivered without complication. Ten out of the 20 prematures had been treated with steroid in the prenatal period; in this case the mother was given 15 mg of dexamethasone intramuscularly once, 47 ± 2.3 h before delivery. The deliveries were vaginal except in two cases where Caesarean section was performed. The 20 newborns were healthy, but of the 20 prematures only 4 were symptom-free. Of the rest, 3 suffered from bronchopneumonia, 4 from HMD I–II, 3 from HMD III–IV,

2 from wet lung syndrome, 1 from hyperviscosity syndrome, and 3 had hyperbilirubinaemia of which 2 needed an exchange transfusion.

The diagnosis of HMD was established on the basis of the clinical signs, X-ray findings, blood gases and the pH [17].

Chemicals

(1,2-^{3}H)-dexamethasone (specific activity 1.48 TB_q/mol) was obtained from the Radiochemical Centre Amersham, UK, unlabelled dexamethasone from Sigma. All other chemicals were obtained from Reanal, Budapest, Hungary.

Binding assays

Determination of dexamethasone binding in whole cells. Blood was separated by Ficoll–Uromiro density gradient centrifugation [4], then washed with Hank's medium. Separated lymphocytes, $1-4 \times 10^6$/tube were incubated in Hank's medium containing ^{3}H-dexamethasone at different concentrations, at 37 °C for 30 min. The amount of non-specifically bound ^{3}H-dexamethasone was determined by incubation in the presence of a 500fold excess of non-labelled dexamethasone. Specific binding was calculated as the difference in radioactivity of samples incubated with and without non-labelled dexamethasone. The number of binding sites per cell was determined using a single saturating concentration of dexamethasone. Dissociation constants were obtained from Scatchard analysis [15] of the data (Fig. 1). The radioactivity was measured with Nuclear Chicago ISOCAP 300 radiospectrofluorometer.

Results

Receptor number per cell was the lowest, 1758 ± 245/cell in, lymphocytes

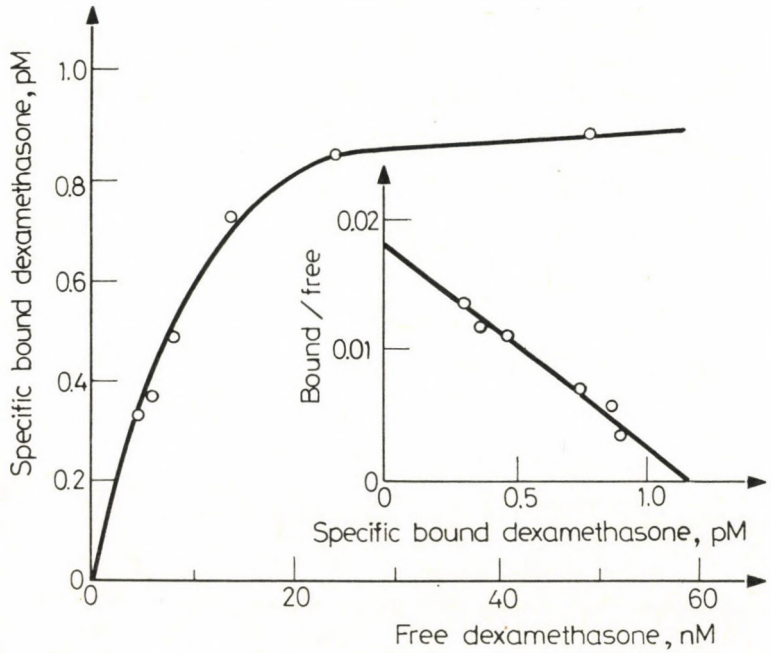

Fig. 1. Determination of dissociation rate constants. Lymphocytes of 4 healthy newborn babies were pooled in order to obtain enough cells for performing saturation analysis. Concentration of dexamethasone varied between 2.5 and 50 nM. The inset shows Scatchard transformation of the data. $K_d = 6.23$ nM

TABLE I

Glucocorticoid receptors in lymphocytes of premature infants

No*	Sex	Gestational age, weeks	Weight at birth	Diagnosis	Steroid receptors per cell	Mean ± SEM
1	M	35	1650	Bronchopneumonia	2772	
2	F	34	2300	Hyperbilirubinaemia	1029	
3	F	33	1900	Bronchopneumonia	2700	
4	M*	33	2005	HMD III–IV	not measurable	
5	F	34	1850	HMD I–II	2304	
6	F	36	2130	HMD II	1324	1446±386
7	F	34	1750	Prematurity	1195	
8	M*	31	1300	HMD III–IV twin A	not measurable	
9	F*	31	1300	HMD III–IV twin B	not measurable	
10	F	36	2050	Prematurity	3132	
11	F	34	1800	HMD II	192	
12	M	26–27	1300	HMD II	734	
13	F	31	1550	Hyperbilirubinaemia	1122	
14	M	32	1950	Hyperbilirubinaemia	4874	
15	M	31	1600	Wet lung syndrome	6528	
16	M	34	1950	Prematurity	1611	2604±726
17	M	36	1950	Bronchopneumonia	1612	
18	F	28	1290	Prematurity	907	
19	F	29	1440	Hyperviscosity syndrome	2600	
20	M	35	1850	Wet lung syndrome	5860	

The number of glucocorticoid receptors in lymphocytes of prematures was determined as described. Nos 1–10: no prenatal steroid administration. Nos 11–20: mothers received 15 mg dexamethasone once 47±2.3 h before delivery. * = died

obtained from the umbilical blood of mature healthy newborns. The mean receptor number of peripheral lymphocytes of mature newborns amounted to 2758±307/cell. The mean receptor number of prematures was 2025±485/cell. Within this, high values were found in babies treated with steroid prenatally: 2604±726/cell, and the lymphocytes of prematures who had not been treated with steroid had only 1446±386 sites/cell. In this latter group specific binding activity was not measurable in 3 cases (Table I). All the three prematures suffered from serious HMD III–IV and died on the 3rd or 4th day of life. Their post mortem findings supported the clinical diagnosis. The mean receptor number in lymphocytes of the 7 prematures, who had not been treated with steroid and survived, was 2064± 256/cell. It was striking that even in babies with steroid therapy a rather low receptor number was observed in three cases. Two of them suffered from HMD II, and one baby was healthy.

DISCUSSION

Ballard and Ballard [1, 2] and Giannopoulos et al. [7, 8] examined the glucocorticoid binding in fetal lung tissues. The presence of high affinity dexamethasone binding sites has been demonstrated in cytoplasmic extracts

from the lung of fetal rats, guinea pigs, rabbits, further from lungs of normal human neonates, but not in lungs of prematures with HMD. In spite of the success of prenatal glucocorticoid treatment in HMD therapy, it cannot be applied in every case because of maternal contraindications. On the other hand, sometimes serious HMD develops in spite of prenatal maternal steroid administration.

The steroid receptors of the lung of living human newborns or prematures cannot be determined, since no tissue samples can be taken. According to Okret et al. [14] the glucocorticoid receptors in different tissues of the same individual are immunologically similar. Their expression might also be related in certain cases. For this reason we examined glucocorticoid binding in peripheral lymphocytes. A mean receptor number of peripheral lymphocytes of mature healthy newborns was 2758 ± 307/cell (K_d: 6.23×10^{-9}M) and a similar number was observed in lymphocytes of premature infants treated prenatally with dexamethasone.

We are not aware of any previous data in the literature on glucocorticoid binding in lymphocytes of newborn and premature infants. According to our results, the receptor content of the lymphocytes of the newborn is slightly lower than that found in adults while their affinity for dexamethasone is about the same [6, 10, 11, 13, 16, 19]. Homo et al. [10] found that the number of steroid receptors and the glucocorticoid sensitivity was dependent on different parameters such as the immunological nature of the cell, the degree of maturation and differentiation, and the stage of proliferation.

We could find no correlation between receptor number and gestational age, birth weight or sex. Ballard and Ballard [2] could not demonstrate specific binding sites in the lungs of prematures who died with HMD. In agreement with this, no specific dexamethasone binding activity could be measured in our three serious HMD cases with fatal outcome. This finding suggests that their lymphocytes either contained no glucocorticoid receptors, or only immature, inactive form. Perhaps a defect in hormone binding may be the cause of the lack of surfactant in the lungs and in turn of the occurrence of HMD. The number of receptors in lymphocytes of the surviving prematures with stage I–II HMD was lower (1131/cell) than that of healthy premature babies or prematures suffering from other diseases.

In our opinion the glucocorticoid binding capacity of lymphocytes may mirror the binding of glucocorticoid in the lung tissue and may possibly be of diagnostic value.

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References

1. Ballard PL, Ballard RA: Glucocorticoid receptors and the role of glucocorticoids in fetal lung development. Proc Nat Acad Sci USA 69:2668, 1972
2. Ballard PL, Ballard RA: Cytoplasmic receptor for glucocorticoids in lung of the human fetus and neonate. J Clin Invest 53:477, 1974
3. Ballard PL, Baxter JD, Higgins SJ, Rousseau GG, Tomkins GM: General presence of glucocorticoid receptors in mammalian tissues. Endocrinology 94: 998, 1974
4. Böyum A: Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21: Suppl. 97, 1968
5. De Sombbre ER: Steroid receptors in breast cancer. New Engl J Med 301:1011, 1979
6. Duval D, Homo F, Thierry C: Glucocorticoid sensitivity of normal lymphocyte populations. In: Glucocorticoid Action and Leukaemia, ed. Bell PA, Borthwick NM, Alpha Omega Publishing Ltd, Cardiff 1979, pp. 143–153
7. Giannopoulos G: Glucocorticoid receptors in lung. I. Specific binding of glucocorticoids to cytoplasmic components of rabbit fetal lung. J Biol Chem 248:3876, 1973
8. Giannopoulos G, Mulay S, Solomon S: Glucocorticoid receptors in lung. II. Specific binding of glucocorticoids to nuclear components of rabbit fetal lung. J Biol Chem 248:5016, 1973
9. Hawkings EF, Hutchens TW, Fligiel S, Horn D, Markland FS: Glucocorticoids and melanoma: receptor properties of dexamethasone sensitive and resistant tumors. J Steroid Biochem 16:673, 1982
10. Homo F, Picard F, Durant S, Gagne D, Simon J, Dardenne M, Duval D: Glucocorticoid receptors and their function in lymphocytes. J Steroid Biochem 12:433, 1980
11. Lippman ME: Glucocorticoid receptors and effects in human lymphoid and leukemic cells. In: Glucocorticoid Hormone Action, ed. Baxter JD, Rousseau GG, Springer Verlag, Berlin 1979, pp. 377–397
12. Munck A, Crabtree GR, Smith KA: Glucocorticoid receptors and actions in rat thymocytes and immunologically stimulated human peripheral lymphocytes. In: Glucocorticoid Hormone Action, ed. Baxter JD, Rousseau GG, Springer Verlag, Berlin 1979, p. 341–355
13. Neifeld JP, Lippman ME, Torney DC: Steroid hormone receptors in normal human lymphocytes. Induction of glucocorticoid receptor activity by phytohemagglutinin stimulation. J Biol Chem 252:2972, 1977
14. Okret S, Carlstedt-Duke J, Wrange Ö, Carlström K, Gustafsson J: Characterization of an antiserum against the glucocorticoid receptor. Biochim Biophys Acta 677:205, 1981
15. Scatchard G: The attractions of proteins for small molecules and ions. Ann N Y Acad Sci 51:660, 1949
16. Schlechte JA, Ginsberg BH, Sherman BM: Regulation of the glucocorticoid receptor in human lymphocytes. J Steroid Biochem 16:69, 1982
17. Schmid F: Paediatrische Radiologie. Springer Verlag, Berlin–Heidelberg–New York 1973
18. Shields JR, Resnik R: Fetal lung maturation and the antenatal use of glucocorticoids to prevent the respiratory distress syndrome. Obstet Gynec 34:343, 1979
19. Simonsson B: Evidence for a glucocorticoid receptor in human leukocytes. Acta Physiol Scand 98:131, 1976
20. Szabó I, Vértes M, Bódis J, Lányi E, Sauerhering E: Steroid receptors in human fetal lung. Lancet 2:751, 1980

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