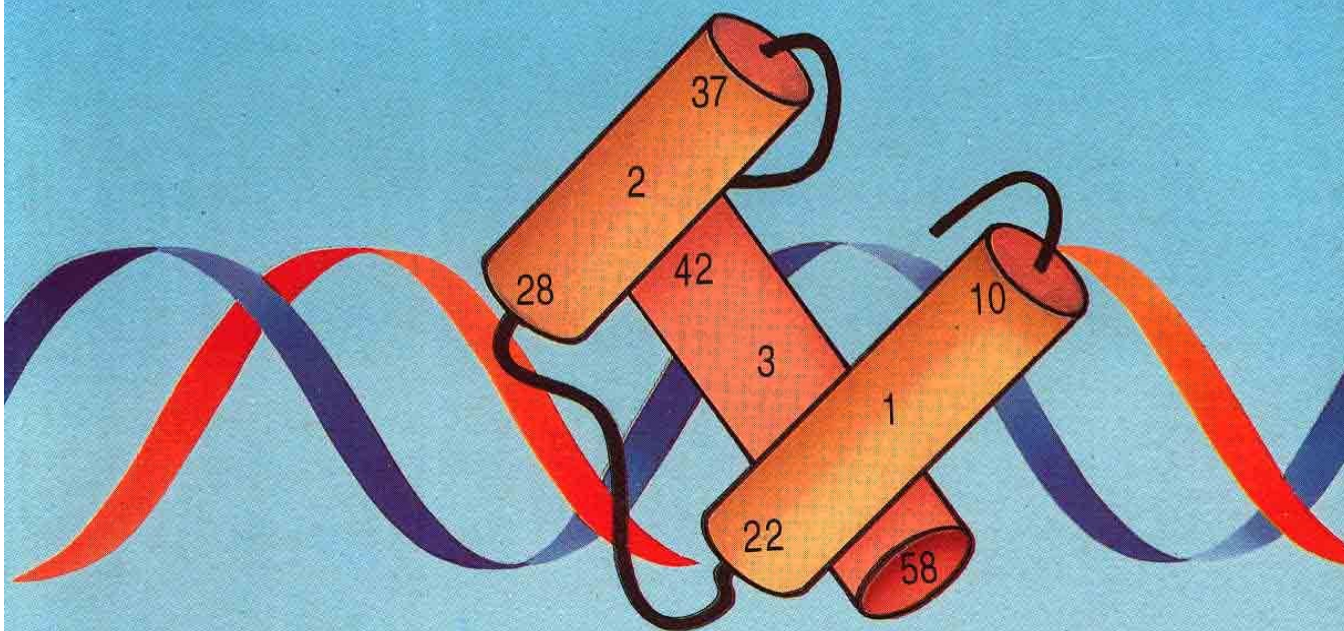


MOLECULAR BIOLOGY OF DEVELOPMENT AND AGING

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Hepatic Glucocorticoid Receptor During Development of Mice: Its Level and Binding to DNA- Cellulose and Nuclei

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SUMMARY

Specific binding of [3H] dexamethasone to its intracellular receptors and the subsequent activation of hormone-receptor complexes were studied in the liver of mice at various postnatal ages. The results indicate that level (fmol/mg protein) of the receptors is significantly higher at 30-day as compared to other ages studied. Scatchard analysis of binding data shows no change in the affinity of hormone to receptor during development. The magnitude of both temperature and salt-dependent activation shows no change in 10 and 60-day-old mice, as judged by DNA-cellulose binding, whereas nuclear binding of temperature activated [3H] dexamethasone-receptor complexes is more pronounced in 10-day-old mice. Cross-mixing experiments show nuclear specificity. DNase I digestion studies showed higher extractability of bound [3H] dexamethasone-receptor complexes from nuclei of 10-day (70%) as compared to 60-day (46%)-old mice. These findings indicate changes in the hepatic glucocorticoid receptor level and in the nature of chromatin organization during development, which might play an important role in the glucocorticoid action in developing animals.

INTRODUCTION

Glucocorticoids are important regulators of cellular development and differentiation. They modulate gene expression through a cascade of regulatory events initiated by high affinity binding to their intracellular receptors (1). The hormone-receptor complexes undergo activation and/or transformation in order to bind to chromatin and affect gene expression. Activation is a multifactorial process, involving dissociation of non-steroid binding proteins (heat shock proteins, Hsp 90) which results in a conformational change in the receptor that exposes the DNA binding domain and hence an increased affinity for nuclear chromatin and/or DNA-cellulose (2). Glucocorticoid receptors are a group of cell specific transacting factors whose activity is controlled by specific binding of the hormone (3). Interaction of activated glucocorticoid-receptor complexes with enhancer sequences (termed as glucocorticoid regulatory elements, GREs usually located upstream from the transcription start site) alter the local configuration of DNA and/or chromatin and modulate the expression of target gene (4).

There are many reports on changes in the glucocorticoid receptor binding sites in different tissues during development and aging (5-7). However, informations on physicochemical changes in the glucocorticoid receptor and/or nuclear chromatin binding are scanty. The present work describes the postnatal changes in the endogenous level of the hepatic glucocorticoid receptor and also the thermal and salt-dependent activation of hormone-receptor complexes. DNase I extraction of bound complexes from liver nuclei of two ages has also been reported.

MATERIALS AND METHODS

Animals and chemicals: Male Swiss albino mice (Balb/c strain), maintained under standard colony conditions, of different postnatal ages (10-, 15-, 30-, 45-, and 60-day) were used. Radiolabeled [3H] dexamethasone (SA, 90 Ci/mmol) was from Amersham, England. Unlabeled dexamethasone and other biochemicals were from Sigma Chemical Co, USA.

Buffers: (A) 0.25 M sucrose/10 mM Tris-HCl, pH 7.5/1 mM EDTA/ 10 mM sodium molybdate/ 10% glycerol/1 mM DTT/10 mM NaCl; (B) 0.25 M sucrose/10 mM Tris-HCl, pH 7.6; (C) 0.25 M sucrose/ 10 mM Tris-HCl, pH 7.6/0.5% Triton X-100.

Preparation and assay of glucocorticoid receptors: Animals were killed by cervical dislocation at a fixed time of the day (1100 h), livers were quickly removed, washed in ice-cold normal saline and blotted dry. Tissues were homogenized in buffer A and centrifuged at 27,500 x g for 60 min at 2°C. Aliquots (100 µl) of clear, fat free cytosol were incubated with 40 nM [3H] dexamethasone alone or with 500-fold excess unlabeled dexamethasone for 4h at 0°C to get the maximum saturable binding. For Scatchard analysis (8), 2.5-120 nM [3H] dexamethasone was used. Dextran-coated charcoal was used to remove any unbound steroid. Specific binding (fmol/mg protein) and the affinity were calculated from maximum saturable binding and Scatchard plot, respectively.

Nuclei and DNA-cellulose binding assays: Liver tissues from two ages (10 and 60-day-old mice) were homogenized in buffer B and centrifuged at 2000 x g for 10 min at 2°C to sediment nuclei. Supernatant thus obtained was further centrifuged at 27,500 x g for 60 min at 2°C. Fat free cytosol was incubated with 40 nM [3H] dexamethasone for 4 h at 0°C; bound hormone-receptor complexes were separated using dextran-coated charcoal. These complexes were then subjected to activation by heat (25°C) and salt (20 mM Ca²⁺ at 0°C) for 45 min. The magnitude of activation was determined by incubating the hormone-receptor complexes with prewashed DNA-cellulose pellets for 60 min at 0°C. DNA-cellulose bound hormone-receptor complexes were obtained by washing the pellets twice with buffer B. The final pellets were suspended in 4 ml of cocktail W (SRL) and counted in a Beckman LS 1801 (efficiency 65% for tritium) for bound radioactivity. For nuclear binding assay, crude nuclei obtained as above was further purified using buffer C and pellets containing 250 µg DNA were obtained. These pellets were incubated with hormone-receptor complexes and processed in the same manner as for DNA-cellulose.

DNase I digestion of nuclei: Temperature activated bound hormone-receptor complexes were extracted from the nuclei using DNase I (150 units/ 100 µg DNA) for 45 min at 0°C. After washing away the extracted hormone-receptor complexes, the pellets were suspended in cocktail and counted for bound radioactivity.

Estimation of protein and DNA: Protein content was determined by the method of Bradford (9) and that of DNA by the method of Burton (10).

RESULTS AND DISCUSSION

Development and aging of animals may partly be characterized by changes in the responsiveness of tissues and cells to certain hormonal signals (11). These hormone-mediated responses are controlled by binding to specific intracellular receptors, by activation of hormone-receptor complexes and translocation of these complexes to nuclear acceptor sites. We report here the changes in hepatic glucocorticoid receptor level as well as in the chromatin organization during development of mice. Our results indicate that the endogenous level (fmol/mg protein) of hepatic glucocorticoid receptor is low at 10- and 15-day of postnatal life, sharply increases at 30-day and thereafter declines in 45- and 60-day-old animals (Fig. 1).

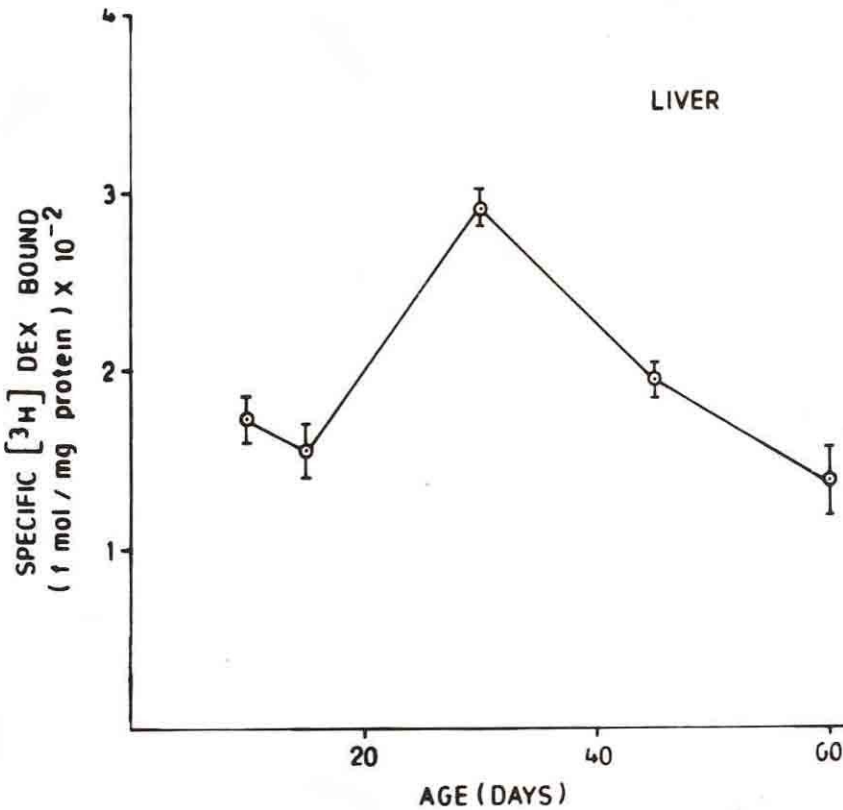


Fig. 1. Specific saturable binding of [3H] dexamethasone in the liver of mice of different postnatal ages. Fractionation and receptor assay conditions are described in Materials and Methods. Values are means for 4-5 mice of each age group. Bars, S.D.

Scatchard analysis of the glucocorticoid receptor binding data confirms a higher level of receptor binding sites at 30-day compared to that of day 15. Slopes of the plots show no change in the affinity of glucocorticoid for its receptors at these two postnatal ages (Fig. 2). Our findings indicate higher level of receptors during weaning period of mice, which may be associated with changing dietary and metabolic adjustment at this phase of postnatal life. The higher level of glucocorticoid receptor protein in the liver of weaning mice may be a contributory factor for the role of this hormone in the growth and development of mice (12). These results are in agreement with earlier reports (5,6), that there is no apparent age-related change in the receptor dissociation constant.

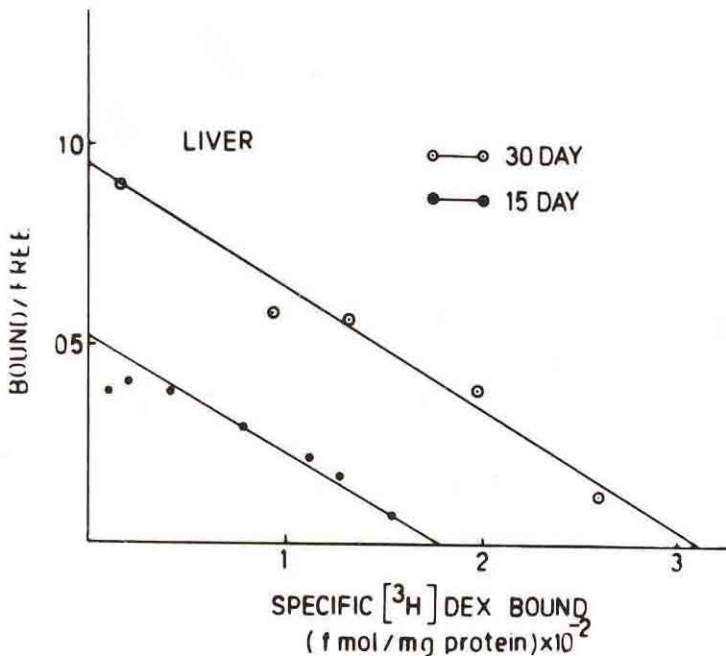


Fig. 2. Scatchard plot of the specific binding of [3H] dexamethasone to liver cytosol of 15- (●) and 30- (○) day-old mice. Curves depict the mean values for two separate assays.

An important aspect of the steroid-induced alteration of gene transcription is the mechanisms by which the steroid-receptor complexes interact with nuclear acceptor sites. Activation of steroid-receptor complexes has been described as involving the dissociation of Hsp 90 from the oligomeric receptor leading to their interaction with DNA sequences modulating gene expression (2). We have studied the activation of glucocorticoid-receptor complexes using DNA-cellulose and purified nuclei from the liver of 10- and 60-day-old

mice to determine the physicochemical changes in receptor molecule at these ages. Result shows that thermal- and salt-dependent activation of glucocorticoid-receptor complexes showed no change in the liver of 10- and 60-day-old mice (Fig. 3), as judged by DNA-cellulose binding of activated hormone-receptor complexes. In contrast to this,

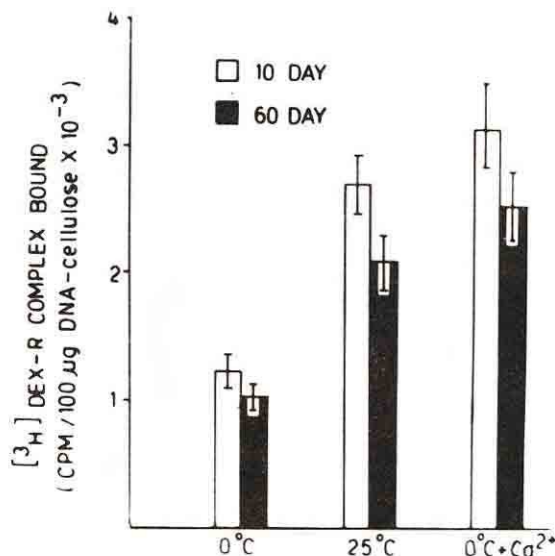


Fig. 3. Specific binding of liver [3H] dexamethasone-receptor complexes to DNA-cellulose in 10- and 60-day old mice. Activation conditions are described in Materials and Methods section. Results are mean + SD for three separate assays with 3-4 mice of each age group.

the nuclear binding of thermally activated glucocorticoid receptor complexes is more pronounced in 10-day-old mice as compared to 60-day (Fig. 4). Cross-mixing experiments showed nuclear specificity. Magnitude of Ca²⁺ dependent activation of hormone-receptor complexes remains unchanged at both the ages even when judged by nuclear binding assay.

DNase I digestion of bound hormone-receptor complexes shows higher (70%) extractability of glucocorticoid receptor from the liver of 10-day as compared to 60-day (46%)-old mice (Fig. 5). This indicates that chromatin conformation is more relaxed allowing higher *in vitro* binding of thermally activated hormone-receptor complexes at day 10 than that of day 60, where chromatin might have acquired more ordered, compact organization. It has earlier been reported that digestibility of chromatin by DNase I decreases as development

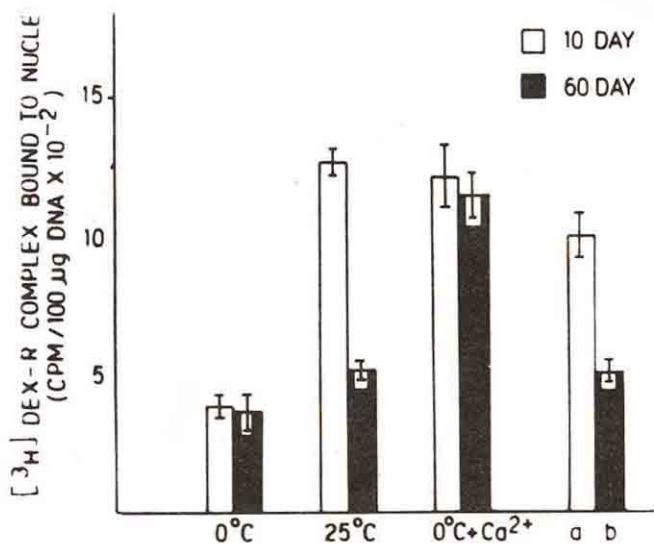


Fig. 4. Specific binding of liver [3H] dexamethasone-receptor complexes to purified nuclei in 10- and 60-day-old mice. Experimental procedures are as mentioned in Materials and Methods. The *a* and *b* barograms represent mixing experiments in which 25°C activated hormone-receptor complexes of 10-day mice incubated with the nuclei of 60-day mice (*a*) and *vice versa* (*b*).

proceeds (13). Our finding corroborates the fact that 10-day-old hepatic nuclear chromatin is more relaxed than that of 60-day-old animal. The efficiency of nuclei to bind estradiol-receptor complexes declines as a function of age (14). The decrease in nuclear binding is correlated with the reduction in nuclear acceptor sites and/or change in the chromatin organization (13). These observations indicate that hepatic glucocorticoid receptor level increases at the weaning period making animals more adaptive to the action of glucocorticoid hormones during that phase of development. Our findings also indicate that chromatin conformation is more relaxed, allowing higher *in vitro* binding of activated hormone-receptor complexes at day 10 as compared to day 60, which might play an important role in the glucocorticoid action mechanism in developing animals.

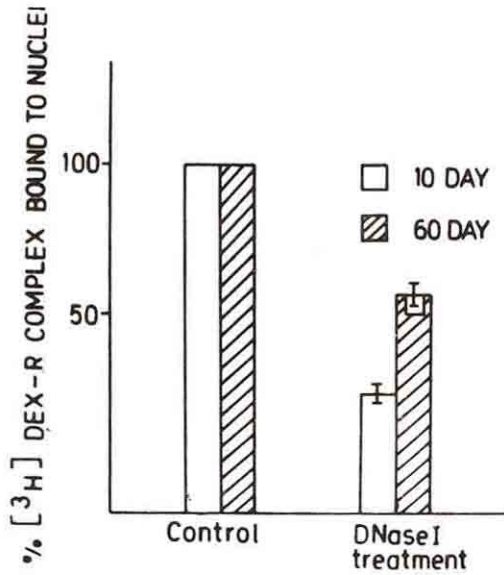


Fig. 5. DNase I extractability of bound [3H] dexamethasone-receptor complexes from the nuclei of 10- and 60-day-old mice. DNase I treatment procedures are given in the text. Values are mean + S.D. of three separate assays.

ACKNOWLEDGEMENTS

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