Indian Journal of Biochemistry & Biophysics
Vol. 36, August 1999, pp. 240-247

Physicochemical characterization of hepatic glucocorticoid receptors from pre- and post-weaned mice

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Received 22 February 1999; revised 17 May 1999

The physicochemical parameters viz., molecular weight, stokes radius and ionic state of hepatic glucocorticoid receptors from pre-(10-day) and post-(60-day) weaned mice were studied. Gel permeation studies of the crude receptors showed a molecular mass of ~290 kDa for the unactivated receptors from both the age groups while the thermally activated receptors showed a molecular mass of ~90 kDa. The stokes radii were ~5.8 and 3.6 for the unactivated and activated receptors, respectively from both the age groups studied. Elution of the bound glucocorticoid receptors from anion-exchanger did not reveal any charge difference in the two age groups; the unactivated receptors eluted at ~250 m*M* KCl whereas the activated receptors eluted at ~100 m*M* KCl. Salt extraction of thermally activated nuclear bound receptors and immunological studies on the unactivated receptors revealed no age-related variation in the two groups of mice. Our findings confirm that the physicochemical properties of hepatic glucocorticoid receptors remain unchanged at these developmental stages of mice.

Glucocorticoids have an active role in metabolic processes, especially those occurring during the developmental period of higher organisms[1]. At cellular level, most known effects of glucocorticoids are mediated by a ~94 kDa intracellular protein, the glucocorticoid receptor (GR). GR belongs to a phylogenetically conserved superfamily of nuclear hormone receptors that include other steroid receptors, thyroid hormone receptors, oncogene products and the recently discovered orphan receptors, whose ligand requirement has not yet been identified[2,3]. This family constitutes the largest known group of eukaryotic transcription factors of which glucocorticoid receptor is the first one to be isolated and studied in detail. The unliganded GR resides in the cytoplasm, where it exists as a large multiprotein complex[4,5]. Upon hormone binding, a conformational change occurs leading to the dissociation of all the receptor associated protein molecules. The hormone-bound receptor then translocates to the nucleus, where binding of the receptors directly to specific response element(s) or to other proteins, e.g., components of AP-1 signaling pathway, lead to modulation of target gene activity[6,7].

The myriad effects of glucocorticoid depend primarily on the intracellular level of receptors[8,9] and also on the steps following hormone binding to receptors (post-receptor events)[10,11]. Moreover, the ontogeny of the glucocorticoid receptors has been correlated to the appearance of glucocorticoid responsiveness in target tissues during development[12-14]. The physicochemical characteristics of the receptor, as development proceeds, is important as any alteration might have a profound influence on the glucocorticoid-mediated signal transduction cascade at each stage of development.

There has been reports, albeit to a limited extent, on the physicochemical properties of glucocorticoid receptors during post-natal development of mice. Kalimi and Gupta[15] have reported changes in the chromatographic profile of rat hepatic glucocorticoid receptors during early stages of development. The same group, however, reported[16] no change in the physicochemical properties between adult (2-6 months) and old (20-24 months) hepatic receptors from Sprague-Dawley rats. In the present study, we have characterized some of the physicochemical properties of the hepatic glucocorticoid receptors from pre- (10-day) and post- (60-day) weaned mice, to gain insight into the developmentally related changes in glucocorticoid responsiveness in target tissues.

Materials and Methods

Materials

Male Swiss albino mice (Balb/c strain) were used. They were housed under normal colony conditions at 24±2°C with a 12 hr light/ dark cycle. Pelleted mice feed (Amrut Laboratories, Pune) and tap water were made available *ad libitum*. [1,2,4,6,7-^{3}H]-

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Dexamethasone (specific activity- 90 Ci/ mmol), a synthetic glucocorticoid, used for the receptor analyses was from Amersham, England. Molecular weight markers, gel permeation media and protein A-Sepharose were obtained from Sigma Chemical Co., USA; anion exchanger (DE-52) was from Whatman. All other chemicals used, were of highest analytical grade.

Buffers of the following composition were prepared in glass double distilled water, the *p*H adjusted at room temperature and stored at 4°C until use.

(A): (i), 100 m*M* potassium phosphate (*p*H 7.5)/1 m*M* EDTA/1 m*M* β-mercaptoethanol; (ii), 100 m*M* potassium phosphate (*p*H 7.5)/1 m*M* EDTA/1 m*M* β-mercaptoethanol/20 m*M* sodium molybdate.

(B): (i), 0.25 *M* sucrose/10 m*M* Tris-HCl (*p*H 7.6); (ii), 0.25 *M* sucrose/10 m*M* Tris-HCl (*p*H 7.6)/0.5% (v/v) Triton X-100.

(C): 10 m*M* potassium phosphate (*p*H 7.5)/1 m*M* β-mercaptoethanol/5 m*M* sodium molybdate.

(D): 0.25 *M* sucrose/10 m*M* Tris-HCl (*p*H 7.5)/1 m*M* EDTA/10 m*M* sodium molybdate/10% (v/v) glycerol/1 m*M* DTT/10 m*M* NaCl.

Tissue preparation

Male mice of two age groups [pre-(10-day) and post-(60-day) weaned] were killed by cervical dislocation at a fixed time of the day (11:00 hr) and the livers were quickly excised, freed of fat and connective tissues, washed twice in ice-cold saline (0.9% NaCl in distilled water) and minced. A 20% (w/v) homogenate of the pooled tissues, of each age group was prepared in buffer A (i), using a motor driven teflon-coated pestle and a glass homogenizer. The homogenates were then subjected to centrifugation at 2,000 × *g* for 10 min at 2°C and the nuclei together with other cellular debris were processed to obtain purified nuclei. The supernatants were further centrifuged at 27,500 × *g* for 60 min at 2°C. The fatty layer on the surface was aspirated using a pasteur pipette and the clear cytosol thus obtained was used for receptor characterization. All the gel filtration and ion-exchange procedures were carried out at 2-4°C, unless otherwise mentioned.

Gel filtration analyses of the unactivated and thermally activated receptors

To the clear, fat free cytosol, prepared in buffer A (i), was added [^{3}H]dexamethasone to a final concentration of 40 n*M*. The contents were mixed by gentle vortexing and incubated on ice for 4 hr . At the end of that period, half the volume of chilled DCC [4% activated charcoal plus 0.4% dextran T-70, prepared in the buffer A (i)] was added and mixed thoroughly by vortexing. After 10 min, the charcoal was pelleted and the supernatants pipetted into ice-cold test tubes. Aliquots of the cytosol, containing [^{3}H]dexamethasone-receptor complexes were incubated for 45 min, either at 2°C to maintain unactivated receptor complexes or at 25°C to give activated receptor complexes[17].

The unactivated complexes, from both the age groups were analysed on a Sephadex G-200 column (1.8 × 45 cm) whereas the activated receptors were analysed on a Sephadex G-100 column (1.8 × 42 cm). Aliquots (2 ml) containing approximately the same radioactivity were loaded onto the column and eluted at a flow rate of 12 ml/hr . Buffer A (ii) was used to elute the Sephadex G-200 column while the same buffer containing 300 m*M* KCl was used for Sephadex G-100 column. Fractions (2 ml) were collected and 100 µl aliquots from each fraction transferred to scintillation vials, 4 ml cocktail-W added and the radioactivity counted for each fraction. Marker proteins of known molecular weight and stokes radius were used to calibrate the respective columns.

The elution volume of [^{3}H]dexamethasone provided the total gel volume (V_p) and that of blue dextran, the void volume (V_o). The distribution coefficient (K_d) and the available distribution coefficient (K_{av}) of the marker proteins and the unactivated receptors were calculated using the following equations:

$$K_d=(V_e-V_o)/(V_i)$$
$$K_{av}=(V_e-V_o)/(V_t-V_o)$$

where, $V_i=V_p-V_o$; V_e, elution volume of the marker proteins and the sample and $V_t=\pi r^2 l$; r is radius of the column and l, length of the gel bed. The apparent molecular weights and the stokes radii of unactivated receptors, from both the ages were calculated according to the theoretical calculations of Andrews[18] and Laurent and Killander[19].

Ion-exchange analysis of unactivated and activated glucocorticoid receptors

To determine the difference in the net charge content of unactivated and thermally-activated glucocorticoid receptors in the two age groups, anion ex-

change chromatography on DEAE-cellulose (DE-52) was done according to the procedure of Grandics *et al.*[20]. A glass syringe of 5 ml capacity, containing a thin film of dextran coated charcoal (~2 mm) was used as the column. The washed resin was then gently poured over the charcoal layer and allowed to settle under pressure to give a gel-bed height of 3 cm. [^{3}H]Dexamethasone-receptor complexes from the liver were prepared in buffer C and 2 ml of it was loaded onto the column. Upon sample application, the column was washed with 30 ml of buffer to remove all the unbound proteins. The bound receptors were subsequently eluted with a 50 ml linear gradient of KCl (0-400 m*M*) in buffer C. Fractions (1 ml) were collected and the radioactivity in 100 µl from each fraction was counted. From the elution plot, concentration of salt at which the unactivated receptor peak eluted was determined. A similar set of experiments provided the data for the thermally activated receptors, except that the receptor complexes were prepared and eluted in buffer C without molybdate.

Salt extraction of the nuclear bound hormone-receptor complexes

Pooled livers from the two age groups of mice were homogenized (20% w/v) in buffer B (i) and centrifuged at 2,000 × *g* for 10 min. The nuclear pellets thus obtained were further processed. To the pellets was added 1 ml of chilled buffer B (ii) and the contents gently homogenized and centrifuged at 2,000 × *g* for 10 min. The supernatants were discarded and the pellets washed thrice in buffer B (i) followed by centrifugation. The final pellets were suspended in the same buffer to give a homogeneous slurry, aliquots of which containing 200-250 µg DNA were pipetted to Eppendorf tubes and 1 ml of buffer B (i) added to each tube. The tubes were centrifuged at 2,000 × *g* for 10 min to give the nuclear pellets, the supernatants being discarded. Aliquots of the activated hormone-receptor complexes containing approximately 30,000 cpm were added in duplicate and the nuclear pellets suspended by gentle vortexing. The nuclear binding was allowed to occur for 1 hr at 2°C with regular mixing of the contents to ensure proper interaction. The amount of nuclear DNA was determined according to the method of Burton[21,22].

Extraction of the hepatic nuclear bound hormone-receptor complexes was done using different concentrations of sodium chloride. The extracting solution (100 µl) contained 0-0.5 *M* NaCl, added from a 1 *M* stock solution prepared in buffer B (i). The extraction was allowed to occur for 45 min at 2°C, with vortexing at regular intervals of time. The reaction was stopped by addition of 1 ml of buffer B (i) and the extracted receptor complexes were removed by washing the pellets, thrice with the same buffer. The control tubes received buffer only and were processed similar to the test samples. Bound radioactivity in the nuclear pellets was then determined.

Immunological studies

Immunological studies were performed with the unactivated glucocorticoid receptors from the liver of 10- and 60-day old mice. The polyclonal antibody was from a synthetic amino acid sequence (SVFSNGYSSPGMRPDVS) corresponding to amino acids 407-423 of the rat glucocorticoid receptor[23]. The unactivated receptors from both the ages were prepared in buffer D, as mentioned above and 200 µl aliquots of cytosols, containing the [^{3}H]dexamethasone-receptor complexes were incubated with 5 µl of either pre-immunized or immunized serum. The incubation was carried out for 18 hr at 2°C. At the end of this period, 50 µl of protein A-Sepharose (25% slurry) was added to each tube and the contents were mixed thoroughly by gentle vortexing. After an incubation period of 1 hr at 2°C, the Sepharose was pelleted by centrifugation at 2,000 × *g* for 10 min. The Sepharose pellets were further washed thrice with ice-cold buffer and the final pellets obtained were suspended in 4 ml of cocktail-W and the bound radioactivity measured.

Results

The results obtained are plotted either as line or bar diagrams, each point representing the mean value ± standard deviation. The results are also presented in tabular form to summarize the data obtained from a series of experiments. All the data generated were statistically analysed and the level of significance (*p*-value) between two sets of data was calculated according to paired student's t-test.

Gel filtration characteristics

Fig. 1A depicts the elution profile of the unactivated [^{3}H]dexamethasone-receptor complexes analysed on a Sephadex G-200 column. The receptors from both the age groups (10- and 60-day) eluted as a single peak at the same elution volume between the standard molecular weight markers, ferritin and β-

amylase. The data were plotted and from the linear regression curves obtained, the molecular mass and the stokes radii (R_s) were calculated. The plot of log M *vs* V_e/V_o showed molecular mass of 285 kDa and 283 kDa for the hepatic glucocorticoid receptors from 10- and 60-day old mice, respectively (Fig. 2A). The stokes radii (R_s), calculated from a plot of $(-\log K_{av})^{1/2}$ *vs* R_s, were found to be 5.81 and 5.76 nm for the unactivated glucocorticoid receptors of 10- and 60-day, respectively (Fig. 3A). The thermally activated [^{3}H]dexamethasone-receptor complexes were analysed on a Sephadex G-100 gel column. The elution profile of the activated receptors showed that the receptors from both the age groups eluted between the markers, alcohol dehydrogenase and bovine serum albumin as a single peak and at the same elution volume (Fig. 1B). A small peak of radioactivity, which eluted in the void volume is probably due to the fraction of receptors remaining in the unactivated state. From the plot of log M *vs* V_e/V_o, the molecular mass were calculated to be 92 kDa (10-day) and 88 kDa (60-day) (Fig. 2B). The stokes radii, calculated from the plot of $(-\log K_{av})^{1/2}$ *vs* R_s gave the values of 3.55 nm (10-day) and 3.52 nm (60-day) (Fig. 3B).

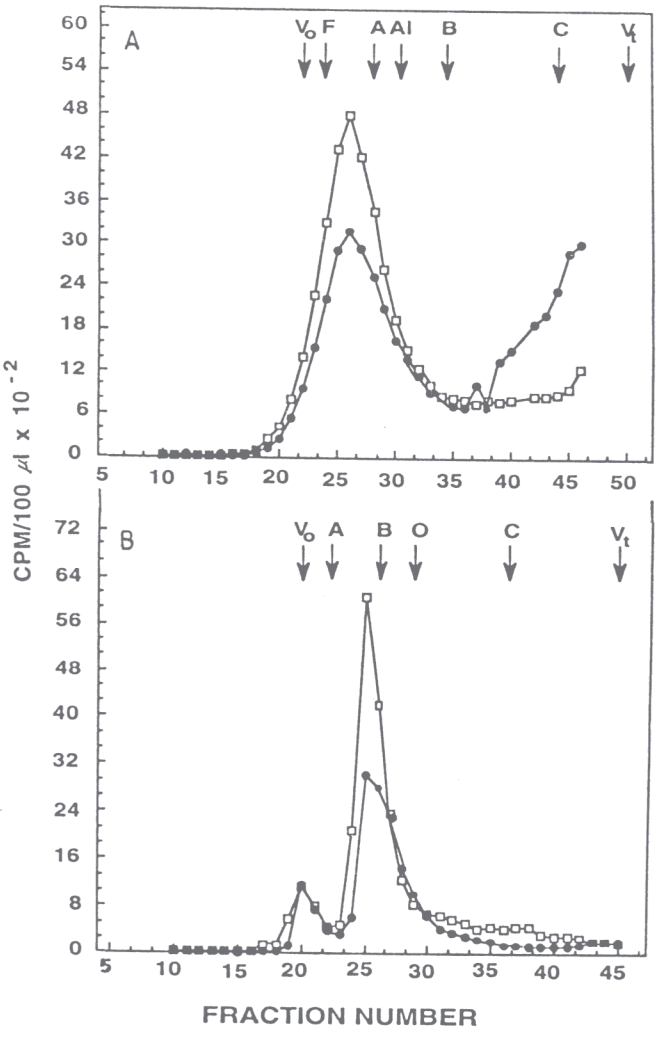

Fig. 1—Gel permeation column chromatography of hepatic unactivated, (A) and activated, (B) glucocorticoid receptors from pre-(10-day) and post-(60-day) weaned mice. [The standard protein markers used were: F, ferritin (443 kDa); A, β-amylase (200 kDa); Al, aldolase (156 kDa); B, bovine serum albumin (67 kDa); O, ovalbumin (45 kDa) and C, cytochrome *c* (12.5 kDa). V_o and V_t, the elution volume of blue dextran and [^{3}H]dexamethasone, respectively. (-□-), 10-day pre-weaned; (-•-), 60-day post-weaned. Each point in the elution profile represents the mean value of three independent experiments conducted from pooled tissues of 3-4 mice of each age group].

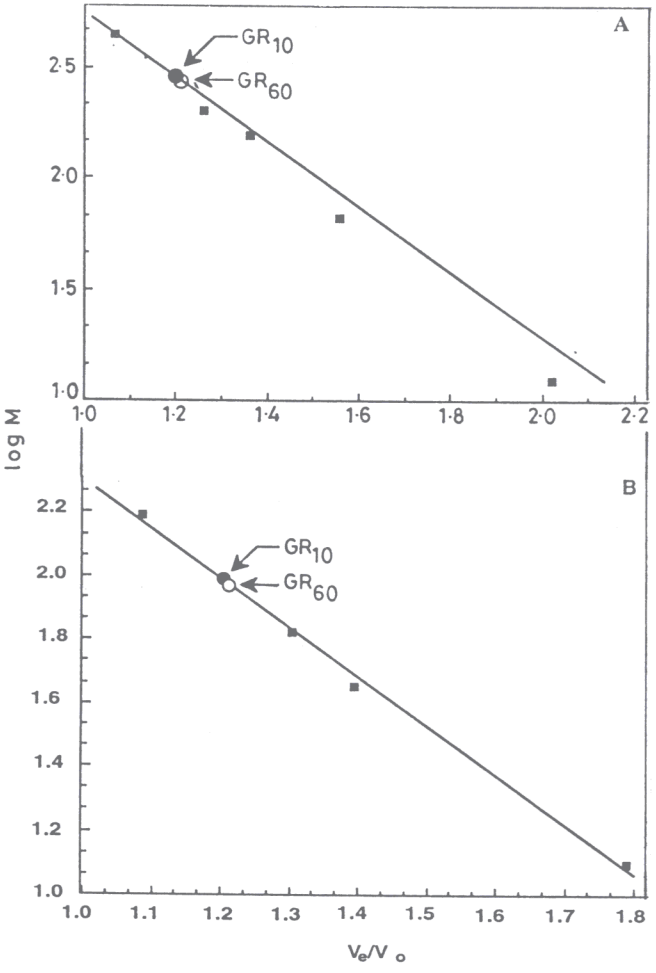

Fig. 2—Plot of log M *vs* V_e/V_o for the determination of molecular mass of unactivated, (A) and thermally activated, (B) glucocorticoid receptors. [The data obtained from respective gel chromatography were plotted to give a linear-regressed curve. GR_{10} and GR_{60} represent the positions of glucocorticoid receptors from 10- and 60-day old mice, respectively. Each point in the curve represents the mean value of three separate experiments. (■), depicts the respective positions of standard protein markers as used in Fig. 1(A) and (B)].

Ion-exchange characteristics

To study the charge difference, if any, in the unactivated as well as activated [^{3}H]dexamethasone-receptor complexes from the liver of 10- and 60-day old mice, ion-exchange characteristics were compared. The elution profile of the unactivated receptors did not reveal any age-related differences in the concentration of salt required to elute the receptors of the two age groups (Fig. 4A). The hormone bound unactivated receptors from both the age groups eluted as a single peak at ~240 m*M* of KCl. Upon thermal activation, the elution of receptors from DE-52 revealed two radioactivity associated peaks, one eluting at ~100 m*M* KCl and the other at ~240 m*M* of KCl (Fig. 4B). The peak which eluted at ~100 m*M* KCl represents the fraction of glucocorticoid receptors that have undergone thermal activation, while the peak at higher salt concentration (~240 m*M*) is contributed by the fraction of receptors that remained unactivated. However, data did not reveal any differences in these parameters at the two ages of mice. The data are also presented in Table1.

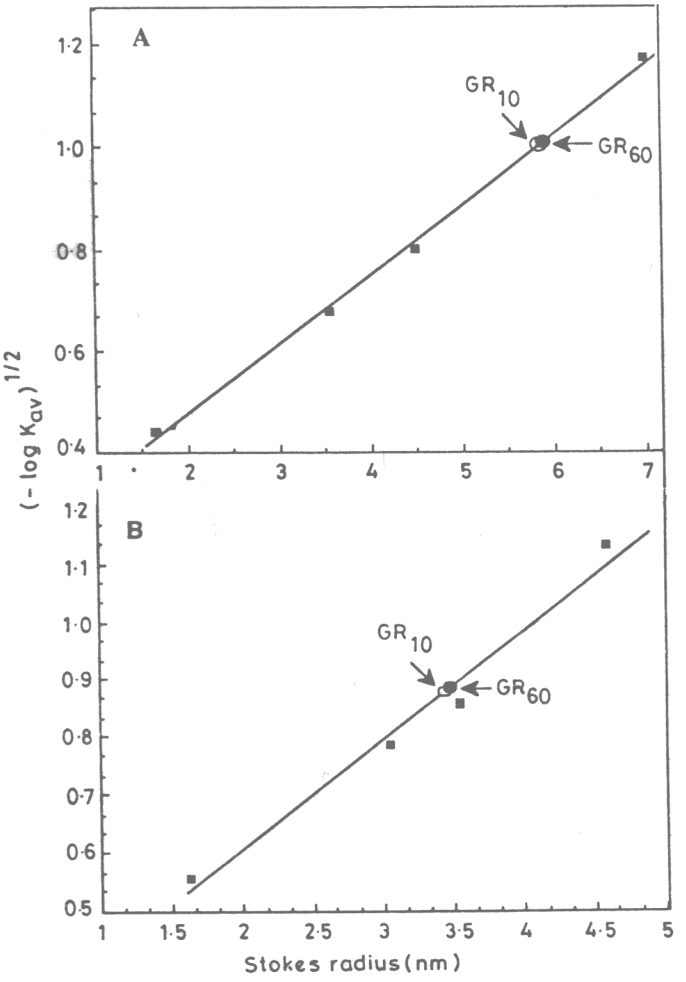

Fig. 3—Plot of $(-\log K_{av})^{1/2}$ *vs* stokes radius for the determination of stokes radii of unactivated, (A) and activated, (B) glucocorticoid receptors. [The plots were obtained from data generated from gel filtration chromatography. The protein markers, (■) of known stokes radius were ferritin (7.0 nm), aldolase (4.5 nm), bovine serum albumin (3.55 nm), ovalbumin (3.05 nm) and cytochrome *c* (1.64 nm). The positions of the receptors from two age groups are indicated as GR_{10} and GR_{60}, for 10- and 60-day old mice, respectively. The values are mean of three experiments].

Salt extraction characteristics

Salt-dependent release of thermally activated, nuclear bound [^{3}H]dexamethasone-receptor complexes from hepatic nuclei also showed no age-associated variations as depicted in Fig. 5. The per cent extraction of the complexes from both the age groups were similar at various concentrations of sodium chloride used. As compared to the control, 50% extraction of the bound receptors occurred at ~0.11 *M* NaCl. Moreover, almost 65% extraction of the bound complexes, from both the age groups occurred at 0.2 *M* NaCl and thereafter the degree of extraction was lowered so much so that ~70-80% extraction was possible only at a very high salt concentration of 0.5 *M* NaCl.

Immunological studies

Differences, if any, in the immunological conformants of the glucocorticoid receptors during postnatal development of mice were examined. The antibody against rat glucocorticoid receptor was able to recognize the mice glucocorticoid receptor. The extent of unactivated [^{3}H]dexamethasone-receptor complexes immunoadsorbed by protein A-Sepharose remained almost similar in the liver of 10- and 60-day old mice (Fig. 6).

Table 1—Physicochemical properties of hepatic glucocorticoid receptor from pre-(10-day) and post-(60-day) weaned mice

Parameters	10-day	60-day
Unactivated receptor		
Molecular mass (kDa)	293 ± 17	286 ± 20
Stokes radius (nm)	5.85 ± 0.04	5.84 ± 0.09
Elution from DE-52 by KCl (m*M*)	245 ± 5	252 ± 4
Activated receptor		
Molecular mass (kDa)	90 ± 2	88 ± 1
Stokes radius (nm)	3.61 ± 0.04	3.58 ± 0.1
Elution from DE-52 by KCl (m*M*)	100 ± 6	112 ± 7

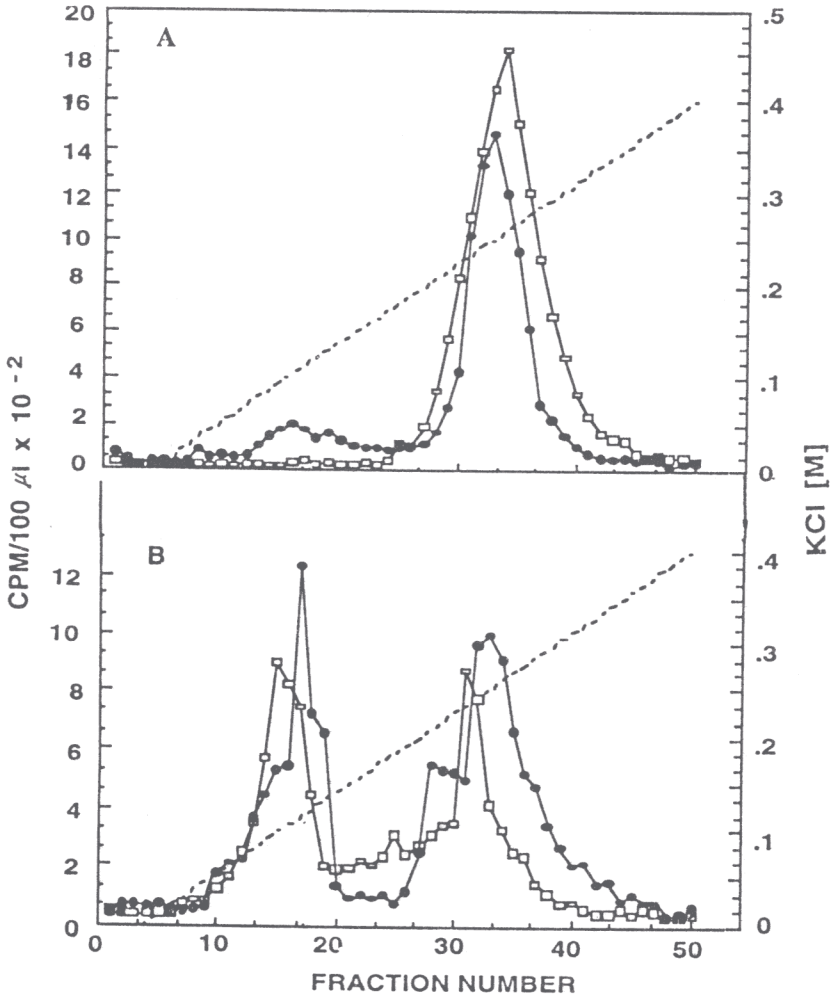

Fig. 4—Elution profile of the hepatic glucocorticoid receptors from anion-exchanger DE-52. [Unactivated and thermally activated [^{3}H]dexamethasone-receptors (500 µl) were loaded onto the column and eluted with a linear gradient of 0-400 m*M* KCl (---). The elution profile of the unactivated receptors is depicted in (A) while that of the activated receptors in (B). (-□-), 10 day pre-weaned; (-•-), 60-day post-weaned. Each point in the two profiles represents the mean value of three independent experiments].

Discussion

Glucocorticoids, acting through their specific intracellular receptors, influence a number of physiologically important processes viz., suppression of immunologic and inflammatory responses, general protein and amino acid metabolism and most importantly they act as a stress-hormone, in response to stressful conditions[24-26]. Glucocorticoid receptors belong to a highly conserved family of nuclear hormone receptors that include receptors for other steroid hormones, thyroid hormones, retinoic acid, oncogene products and orphan receptors[3]. Changes in adaptive response to hormonal stimuli are characteristic of developing animals[27]. There are many reports on the age-regulated alterations in the mechanism of glucocorticoid action that is reflected among others, by a decreased ability with age, to induce many hepatic enzymes and by a potential association of many pathophysiological states with tissue-/age-specific and/or acquired glucocorticoid resistance or hypersensitivity[6,28]. This decreased responsiveness may either be due to a decline in the receptor concentration or changes in the events following hormone binding to receptors (post-receptor events). Moreover, the internal milieu of a cell is likely to undergo changes, with development that may affect the physical and functional attributes of the glucocorticoid-mediated signal transduction processes.

Earlier studies from this laboratory have reported changes in the glucocorticoid receptor level, with post-natal development in the liver and kidney of mice[29,30]. Also, the nuclear binding of thermally acti-

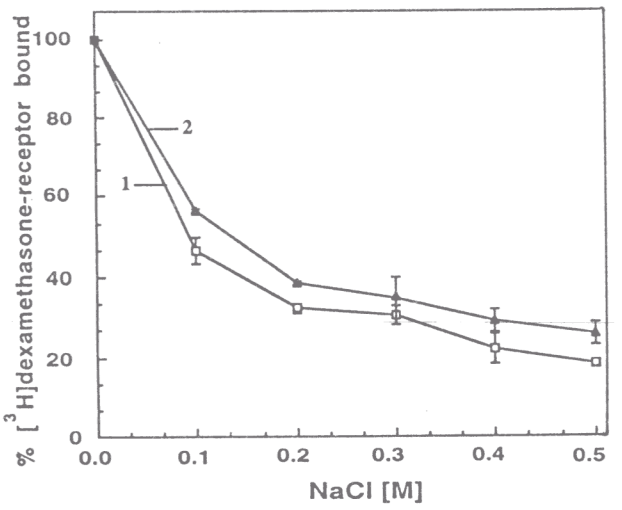

Fig. 5—Extraction of the nuclear bound [^{3}H]dexamethasone-receptor complexes by salt. [Thermally activated, hepatic nuclear bound receptor complexes were extracted using increasing concentrations of NaCl for 45 min at 2°C. Curve 1, 10-day pre-weaned; curve 2, 60-day post-weaned. The values are mean ± standard deviation of three independent experiments].

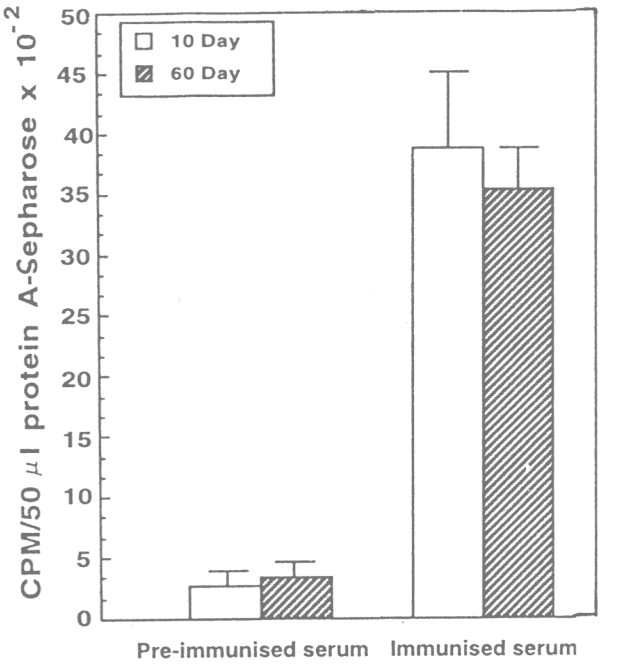

Fig. 6—Immuno adsorption of glucocorticoid receptors by protein A-Sepharose. Hepatic unactivated [^{3}H]dexamethasone-receptor complexes (200 µl) were incubated with 5 µl of either preimmunised or immunized serum for 18 hr at 2°C, followed by addition of 50 µl of protein A-Sepharose. After 1 hr, the Sepharose was pelleted, washed thrice, suspended in cocktail and the bound radioactivity measured. The results are mean ± standard deviation of three independent experiments].

vated receptors, from the same tissues, showed a difference at these two ages (10- and 60-day)[29,30]. The difference was attributed primarily to the changes in the chromatin organization that allow greater binding of the activated receptors at early post-natal ages of mice. Our present study aimed at understanding changes, if any, in the physicochemical parameters of the hepatic glucocorticoid receptor at two ages (pre- and post-weaned) during post-natal development of mice. Gel permeation analyses indicate that the hepatic glucocorticoid receptors eluted as a single peak from both the ages of mice. The molecular mass and stokes radii of the unactivated and thermally activated glucocorticoid receptors are in general agreement with the values reported earlier for the crude as well as purified receptors from the liver and other tissues of rats[16,20].

Our findings also indicate no charge differences in the unactivated as well as thermally activated glucocorticoid receptors, from the two post-natal ages of mice, as evident from the elution pattern from anion-exchanger. Moreover, the concentrations of salt required to elute the unactivated as well as activated receptors are similar to that reported by other workers for the rat liver glucocorticoid receptors[31,32]. Our earlier work did reveal a difference in the degree of activated receptor binding to nuclear DNA at these two ages, that was shown to be due to developmentally related changes in the chromatin organization to a higher ordered structure[29,30]. To check if there was any alteration in the binding affinity of the receptors to chromatin, primarily through ionic interactions, salt extraction of the bound receptor complexes from hepatic nuclei was done. Data did not reveal any age-related differences in the percentage of extraction at various salt concentrations used. About 70-80% extraction could be achieved even at a high salt concentration of 0.5 *M* NaCl from both the age groups. This suggests that a small percentage of the activated receptors is involved in high affinity (specific) binding whereas, a major fraction is involved in low affinity (non-specific) interaction with nuclear chromatin. Non-specific DNA-binding facilitates the interaction of trans-acting factors to specific sequences and this probably acts as a buffer to prevent saturation of a small number of high affinity sites, thereby preventing full induction or repression of cognate genes over a narrow concentration of hormone[33,34].

The high affinity of the anti-rat GR antibody to the mice glucocorticoid receptor, reflects a high degree of

structural homology as far as the immunologic domain is concerned. Furthermore, the immunoprecipitation and adsorption studies showed no variation in the immunological conformant of the glucocorticoid receptor during pre- and post-weaned post-natal age of mice. Taken together, these findings reveal no change, *per se* , in the physical and chemical properties of the hepatic glucocorticoid receptor during pre- and post-weaned stages studied.

Acknowledgement

This work was supported by Grant-in-aid (No. SP/SO/D-35/89) from Department of Science & Technology, New Delhi. MAB thanks the Department of Biochemistry, NEHU, Shillong for providing the facilities. The polyclonal anti-rat-GR antibody gift from Professors N Katunuma and H Kido, Institute for Enzyme Research, Tokushima University, Japan is gratefully acknowledged.

References

1 Evans R M (1988) *Science* 240, 889-895
2 Laudet V, Begue A, Henry-Duthiot C, Joubel A, Martin P, Stehelin A & Saule S (1991) *Nucleic Acids Res* 19, 1105-1112
3 Mangelsdorf D J, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P & Evans R M (1995) *Cell* 83, 835-839
4 Pratt W B (1993) *J Biol Chem* 268, 21455-21458
5 Czar M J, Owens-Grillo J K, Dittmar K D, Hutchison K A, Zacharek A M, Leach K L, Deibel M R & Pratt W B (1994) *J Biol Chem* 269, 11155-11161
6 Bamberger C M, Schulte M & Chrousos (1996) *Endocrine Rev* 17, 245-261
7 Barrett T J, Vig E & Vedeckis W V (1996) *Biochem* 35, 9746-9753
8 Vanderbilt J N, Meisfeld R, Maler B A & Yamamoto K R (1987) *Mol Endocrinol* 1, 68-74
9 Pepin M C, Pothier F & Barden N (1992) *Nature, London* 355, 725-728
10 Sharma R (1993) *Curr Sci* 65, 342-347
11 Roth G S (1985) in *Homeostatic Functions and Aging* (Davis B B & Wood W G eds), pp 41-58, Raven Press, New York
12 Feldman D (1974) *Endocrinol* 95, 1219-1227
13 Cole T J, Blendy J A, Monaghan A P, Krieglstein K, Schmid W, Unsicker K & Schutz G (1995) *Genes Dev* 9, 1608-1621
14 King L B, Vacchio M S, Dixon K, Hunziker R, Margulies D H & Ashwell J D (1995) *Immunity* 3, 647-656
15 Kalimi M & Gupta S (1982) *J Biol Chem* 257, 13324-13328
16 Kalimi M , Gupta S, Hubbard J & Greene K (1983) *Endocrinol* 112, 341-347
17 Sharma R & Timiras P S (1987) *Biochim Biophys Acta* 930, 237-343
18 Andrews P (1970) in *Methods in Biochemical Analysis* (Glick D ed), Vol. XVIII, pp 1-53, John Wiley & Sons, New York
19 Laurent T C & Killander J (1964) *J Chromatogr* 14, 317-330
20 Grandics P, Miller A, Schmidt T J, Mittman D & Litwack G (1984) *J Biol Chem* 259, 3173-3180
21 Burton K (1956) *Biochem J* 62, 315-322
22 Burton K (1968) *Methods Enzymol* 12B, 163-166
23 Sharma R, Kido H & Katunuma N (1991) *Biochem Med Metab Biol* 46, 246-254
24 Baxter J D & Forsham P F (1972) *Am J Med* 53, 573-589
25 Sapolsky R M, Krey L C & McEwen B S (1986) *Endocrine Rev* 7, 284-293
26 Norman A W & Litwack G (1987) in *Hormones* (Norman A W & Litwack G eds), pp 263-320, Academic Press
27 Roth G S (1989) in *Endocrine Function and Aging* (Armbrecht H J ed), Springer-Verlag, New York
28 Sharma R (1988) in *Physiological Basis of Aging and Geriatrics* (Timiras P S ed), pp 75-86, Macmillan Press, New York
29 Borbhuiya M A & Sharma R (1995a) *Biochem Mol Biol Intl* 37, 645-652
30 Borbhuiya M A & Sharma R (1995b) *Indian J Biochem Biophys* 32, 125-129
31 Schmidt T J, Miller-Diener A, Webb M L & Litwack G (1985) *J Biol Chem* 250, 16255-16262
32 Bodine P V & Litwack G (1988) *J Biol Chem* 263, 3501-3512
33 Winter R B & von Hipple P H (1981) *Biochemistry* 20, 6948-6960
34 Cavanaugh A H & Simmons S S, Jr (1990) *Biochemistry* 29, 989-1002