*Developmental Brain Research*, 36 (1987) 285–287
Elsevier

BRD 60234



# Age-dependent activation of glucocorticoid receptors in the cerebral hemispheres of male rats

Ramesh Sharma* and Paola S. Timiras

*Department of Physiology-Anatomy, University of California, Berkeley CA 94720 (U.S.A.)*

(Accepted 28 July 1987)

*Key words:* Glucocorticoid receptor; Activation; Cerebral hemisphere; Age

The binding of [$^3$H]dexamethasone-receptor complexes to purified nuclei was studied in the cerebral hemispheres of immature (3-week-old) and mature (26-week-old) Long–Evans male rats to determine the age-related changes, if any, in the physicochemical properties of glucocorticoid receptors. Our data show that heat activation (for 45 min at 25 °C) significantly enhances the nuclear binding of [$^3$H]dexamethasone-receptor complexes in rats of both ages, with a greater magnitude in immature rats. $Ca^{2+}$ activation (20 mM $Ca^{2+}$ for 45 min at 0 °C) also enhances the nuclear binding of bound receptor complexes but to a similar extent at both ages. These findings indicate that some of the physicochemical properties (e.g. heat activation) of glucocorticoid receptor change, while others (e.g. $Ca^{2+}$ activation) remain unchanged at different phases of the lifespan.

Responses to stress depend on the age of the animal and are mediated through glucocorticoids which act on neural tissues by binding to intracellular receptors followed by activation of bound receptor complexes[18]. The activation of steroid-receptor complexes has been described as a not well-defined, conformational change which enables the complexes to interact with specific acceptor sites on chromatin and modulate gene expression[22]. This nuclear binding capacity can be achieved in vitro by incubating the hormone-receptor complexes at 25 °C[11,14] and under high ionic conditions[1,9]; this action is achieved possibly by exposure of positively charged amino acid residues on the surface of the receptor molecule which in turn enhances the affinity for nuclei and polyanions such as DNA[6,14]. The activation of the glucocorticoid receptor occurs in vivo under physiological conditions and is thought to be rate limiting for nuclear binding[13,15].

Adaptive responsiveness to hormones are age-related phenomena as are changes in induction of many enzymes[12]. These hormone-mediated responses are controlled by binding of the hormone to specific intracellular receptors, by activation of hormone-receptor complexes and interaction of these complexes to nuclear acceptor sites. There are many reports on the quantitative changes in the brain glucocorticoid receptors during development and aging[10]. However, there is little information on qualitative changes in brain receptors as a function of age. In the present communication, we report a significant difference in the thermal activation of glucocorticoid receptors in the cerebral hemispheres of immature (weanling) and mature rats.

Immature (3-week-old, just weaned) and mature (26-week-old adult) Long–Evans male rats, maintained at 24 ± 2 °C on a 12/12 h light/dark period, were fed Purina rat chow pellets and water ad libitum. The animals were bilaterally adrenalectomized and were given 0.9% NaCl instead of water for 3 days following adrenalectomy. All the chemicals used were of analytical grade, and biochemicals were purchased from Sigma U.S.A. [1,2,4,6,7-$^3$H]Dexamethasone (spec. act. 78.7 Ci/mmol) was obtained from

* Permanent address: Department of Life Sciences, Manipur University, Imphal 795003, India.
*Correspondence:* P.S. Timiras, Department of Physiology-Anatomy, University of California, Berkeley, CA 94720, U.S.A.

Amersham with radiochemical purity of 96.1% by HPLC.

The rats were killed by decapitation at a fixed time of day (11.00 h) and brains were quickly removed, blotted free of blood and superficial blood vessels. Cerebral hemispheres were minced and homogenized in 4 vols. (w/v) of TS buffer (10 mM Tris-HCl/0.32 M sucrose, pH 7.5) at 0 °C using a Teflon homogenizer. The homogenates were first centrifuged at 1000 *g* for 10 min at 0 °C to sediment nuclei. The resulting supernatants were further centrifuged at 105,000 *g* for 60 min at 0 °C. The clear fat free cytosols were incubated for 2 h at 0 °C with 40 nM [$^3$H]dexamethasone alone or with excess of non-radioactive dexamethasone. Free hormones were removed by adding dextran coated charcoal (3% charcoal, 0.3% dextran T-70 in TS buffer). The clear supernatants were used for nuclear binding assays.

The crude nuclear pellets were purified[5] using 0.5% Triton X-100 in TS buffer. The resulting nuclei were washed thrice with TS buffer and finally suspended in the same buffer. Aliquots of nuclear suspension containing 50–75 $\mu$g of DNA were centrifuged at 2000 *g* for 10 min at 0 °C and the supernatant fractions were discorded. [$^3$H]Dexamethasone-labeled cytosol (200 $\mu$l) was added in duplicate to above nuclear pellets. The samples were gently mixed on a vortex and incubated at 0 °C for 1 h. At the end, 1.0 ml of cold TS was added and nuclei were pelleted and washed twice. The nuclear pellets were finally suspended in 0.5 ml of TS and transferred to vials containing 4.0 ml of complete counting Cocktail (3a70B) obtained from Research Products International, IL. The radioactivity was counted in a Beckman LS-100C liquid scintillation counter with an efficiency of 51.5% for tritium. DNA contents were determined[2] and the data were statistically analyzed[7].

Development and aging of animals may partly be characterized by changes in responsiveness of tissue and cells to certain hormonal modulators[3]. The occurrence of quantitative changes in receptor molecules is well documented[10,16]. The possibility that qualitative changes occur as well is still uncertain. Our data indicate the presence of such qualitative changes. The heat activation significantly enhances the nuclear binding of [$^3$H]dexamethasone-receptor complexes in rats of both ages with a greater magnitude in immature (53%) than mature rats (Fig. 1). The nuclear binding of hormone-receptor complexes at 0 °C is similar at both ages. These findings are in agreement with the age-dependent decline in thermal activation of rat uterine estradiol receptors[4]. It has been reported that the nuclear binding of glucocorticoid-receptor complexes reaches mature levels only after day 10 in the pituitary of rats. The early reduced transfer of hormone-receptor complexes to nuclei is correlated with the non-responsive period to stress in neonatal rats[17]. The higher degree of activation of glucocorticoid receptors at weaning ages may be a contributory factor in a greater role of glucocorticoids in the adaptive response to stress at this phase of the life span[18]. Interestingly, rat skeletal muscle glucocorticoid-receptor complexes do not show age-related differences in thermal activation[19]. Recently, we have observed a greater heat activation of [$^3$H]-dexamethasone-receptor complexes in the liver of

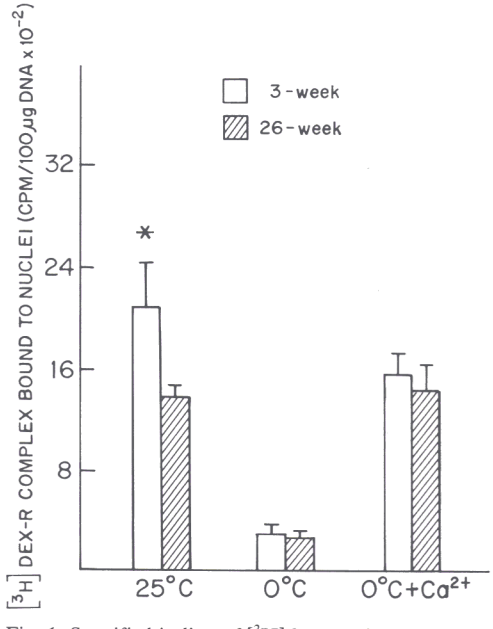


Fig. 1. Specific binding of [$^3$H]dexamethasone-receptor (DEX-R) complexes to cerebral hemisphere nuclei from immature (3-week-old) and mature (26-week-old) male rats. Cytosols were incubated with 40 nM [$^3$H]dexamethasone in the presence or absence of excess unlabeled dexamethasone for 2 h at 0 °C. The cytosols were further incubated at: (i) 25 °C for 45 min; (ii) 0 °C for 45 min; and (iii) 0 °C with 20 mM $Ca^{2+}$ for 45 min. Following incubation, specific nuclear binding was determined as mentioned in materials and methods. The results are mean ± S.D. for 3 separate experiments with 4 or 5 rats of each age group. * Statistically significant ($P < 0.02$) with respect to 26-week.

mature rats as compared to immature ones[20]. These findings indicate tissue-specific changes in the physicochemical properties of glucocorticoid receptors. In addition, $Ca^{2+}$ activation significantly enhances the nuclear binding of bound receptor complexes in the cerebral hemispheres of rats to the same degree at both ages (Fig. 1). We have reported earlier that $Ca^{2+}$-dependent low temperature activation of glucocorticoid-receptor complexes decreases in rat skeletal muscle[19] while it remains unchanged in the liver[20] of mature rats as compared to immature ones. This differential activation of glucocorticoid receptor in different tissues of rats supports the concept of receptor polymorphism and production of different responses in various tissues[21]. The exact mechanisms of this low temperature $Ca^{2+}$ activation of the glucocorticoid-receptor complexes are not well understood. However, it may be due to the direct interaction of $Ca^{2+}$ with the receptor molecule and/or receptor transforming factor(s). This interaction could cause a conformational change capable of exposing the DNA- and chromatin-binding domain[8].

The present findings indicate that some of the physicochemical properties, such as heat activation of glucocorticoid receptor change, while others, such as $Ca^{2+}$ activation, remain unchanged in the cerebral hemispheres of immature and mature rats. The observed differences in glucocorticoid receptors may lead to functional changes in the tissue response as a function of age.

R.S. offers thanks to the Ministry of Human Resources and Development, Government of India for National Scholarship.

1 Bailly, A., Savouret, J.F., Sallas, N. and Milgrom, E., Factors modifying the equilibrium between activated and non-activated forms of steroid-receptor complexes, *Eur. J. Biochem.*, 88 (1978) 623–632.
2 Burton, K., A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, *Biochem. J.*, 62 (1956) 315–323.
3 Cake, N. and Litwack, G., The glucocorticoid receptor. In G. Litwack (Ed.), *Biochemical Actions of Hormones, Vol. III,* Academic, New York, 1976, pp. 317–390.
4 Chuknyiska, R.S., Justiniano, C. and Roth, G.S., Impaired conversion of rat uterine estradiol receptors during aging, *Exp. Gerontol.*, 21 (1986) 255–265.
5 Eberhardt, N.L., Valcana, T. and Timiras, P.S., Triiodothyronine nuclear receptors: an in vitro comparison of the binding of triiodothyronine to nuclei of adult rat liver, cerebral hemispheres, and anterior pituitary, *Endocrinology,* 102 (1978) 556–561.
6 Disorbo, D.M., Phelps, D.S. and Litwack, G., Chemical probes of amino acid residues affect the active sites of the glucocorticoid receptors, *Endocrinology,* 106 (1980) 922–929.
7 Garrett, H.E., *Statistics in Psychology and Education,* David Makay Company, New York, 1966.
8 Grody, W.W., Schrader, W.J. and O'Malley, B.W., Activation, transformation, and subunit structure of steroid hormone receptors, *Endocrinol. Rev.*, 3 (1982) 141–163.
9 Higgins, S.J., Rousseau, G.G., Baxter, J.D. and Tomkins, G.M., Early events in glucocorticoid action, *J. Biol. Chem.*, 248 (1973) 5866–5872.
10 Kalimi, M., Glucocorticoid receptors: from development to aging. A review, *Mech. Ageing Dev.*, 24 (1984) 129–138.
11 Kalimi, M., Beato, M. and Feigelson, P., Interation of glucocorticoids with rat liver nuclei. I. Role of cytosol proteins, *Biochemistry*, 12 (1973) 3365–3371.
12 Kanungo, M.S., Changes in enzymes during ageing. In *Biochemistry of Ageing,* Academic, London, 1980, pp. 79–126.
13 Markovic, R.D. and Litwack, G., Activation of liver and kidney glucocorticoid-receptor complexes occurs in vivo, *Arch. Biochem. Biophys.*, 202 (1980) 374–379.
14 Milgrom, E., Atger, M. and Baulieu, E.E., Acidophilic activation of steroid hormone receptors, *Biochemistry,* 12 (1973) 5198–5205.
15 Munck, A. and Foley, R., Activation of steroid hormone-receptor complexes in intact target cells in physiological conditions, *Nature (London),* 278 (1979) 752–754.
16 Roth, G.S. and Hess, G., Changes in the mechanisms of hormone and neurotransmitter action during aging: current status of the role of receptor and post-receptor alterations, *Mech. Ageing Dev.*, 20 (1982) 175–194.
17 Salky, M. and Koch, B., Ontogenesis of glucocorticoid receptors in anterior pituitary gland: transient dissociation among cytosolic receptor density, nuclear uptake and regulation of corticotropic activity, *Endocrinology,* 108 (1981) 591–596.
18 Sapolsky, R.M. and Meaney, M.J., Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period, *Brain Res. Rev.*, 11 (1986) 65–76.
19 Sharma, R. and Timiras, P.S., Regulatory changes in glucocorticoid receptors in the skeletal muscle of immature and mature male rats, *Mech. Ageing Dev.*, 37 (1987) 249–256.
20 Sharma, R. and Timiras, P.S., Age-dependent activation of glucocorticoid receptors in the liver of male rats, *Biochem. Int.*, 13 (1986) 609–614.
21 Webb, M.L., Miller-Diener, A.S. and Litwack, G., Purification, characterization, and activation of the glucocorticoid-receptor complex from rat kidney cortex, *Biochemistry,* 24 (1985) 1946–1952.
22 Yamamoto, K.R.A., Steroid receptor regulated transcription of specific genes and gene networks, *Annu. Rev. Genet.*, 19 (1985) 209–252.