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CHANGES IN GLUCOCORTICOID RECEPTORS IN DIFFERENT REGIONS OF BRAIN OF IMMATURE AND MATURE MALE RATS

Ramesh Sharma* and Paola S. Timiras#

Department of Physiology-Anatomy, University of California, Berkeley, California 94720, USA

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Summary. Specific cytosolic binding for synthetic glucocorticoid dexamethasone was studied in several brain regions (hypothalamus, hippocampus, caudate nucleus, cerebellum, cerebral cortex) of immature (3-week) and mature (26-week) male rats, intact and adrenalectomized. A significant regional difference was observed in the concentration of *in vitro* [^{3}H] dexamethasone binding in the brain of adrenalectomized rats at both ages, with the highest levels in the hippocampus. A marked decrease in specific binding was observed in all brain regions of adrenalectomized mature rats as compared to immature. The dexamethasone binding was significantly lower in all brain regions of normal intact animals as compared to adrenalectomized rats in both ages.

Introduction

Corticosteroid hormones appear to exert a multitude of effects on the nervous system. These effects range from regulation of the most basic processes of cellular growth and differentiation, to alterations in electrophysiological activity, and finally influences on mood, motivation, and learned behavior patterns (1). The full elucidation of such effects requires a thorough understanding of corticosteroid action at the molecular level. The actions of hormones are crucial in regulating most metabolic functions. Many of these functions, and overall maintenance of homeostasis are altered with age (2). Rat brain glucocorticoid receptors are detectabl as early as the 17th day of gestation. After birth, the receptor-binding capacity gradually increases to adult levels by 15 days of age (3). An age related decline in brain cytosolic glucocorticoid binding sites has been reported in aged (24-months) rats (4). Glucocorticoid-binding proteins in various regions of the brain of different animals have been reported (5,6). A site specific decrease in glucocorticoid receptors in the aged rat brain

* Permanent address: Department of Life Sciences, Manipur University, Canchipur, Imphal-795003, India.
* Under National Scholarship from Government of India.
# To whom correspondences should be addressed.

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has been observed (7). It has been reported that the hippocampus of the rat brain progressively loses corticosterone receptors with age (8). The receptor loss is attributed to decreased concentration of cytosolic corticosterone receptors, with no change in receptor affinity or capacity for nuclear translocation (8). Studies on glucocorticoid binding in various brain regions of different animals have demonstrated that the hippocampus is the primary glucocorticoid concentrating region of the brain (9,10,11). Nelson et al. (12) reported a significant regional difference with no effect of age on cytosolic corticosterone binding in male C57BL/6J mice. There are many variable reports on the glucocorticoid receptors in the brain during development and aging of animals. The present paper describes the in vitro specific dexamethasone binding sites in different brain regions of reproductively immature and mature male rats to elucidate certain molecular mechanisms of glucocorticoid regulation in different regions of brain as a function of age.

MATERIALS AND METHODS

Animals. Immature (3-week) and mature (26-week) Long-Evans male rats, maintained at 24±2°C with a 12h light period followed by a 12h dark period, were used. The rats were fed Purina 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.

Chemicals. All the chemicals used were of analytical grade, and biochemicals were purchased from Sigma chemical Co., USA. [1,2,4,6,7-^{3}H] dexamethasone (specific activity 82.4 Ci/mmol) was obtained from Amersham with radiochemical purity of 95% by HPLC. Nonradioactive dexamethasone was purchased from Sigma. Whatman glass microfibre filters (GF/A) were obtained from Fisher Scientific Co. USA. Complete counting cocktail (3a70B) was purchased from Research Products International Corporation, Illinois.

Tissue and cytosol Preparation. The rats were killed by decapitation at a fixed time of the day (1PM). The brains from intact and adrenalectomized rats were quickly removed, blotted free of blood and superficial blood vessels. Different parts of the brain (hypothalamus, hippocampus, caudate, cerebellum, cerebral cortex) were carefully dissected on an ice-chilled stage and stored in separate vials at -70°C. Brain regions from 4-5 rats were pooled and homogenized in 5 volume (W/V) of TEGBN040 buffer (10 mM Tris-HCl, pH 8.1/1mM Na_2EDTA/10% glycerol/1 mM 2-mercaptoethanol/100 µg of crystalline bovine serum albumin per ml/200 µM phenylmethylsulfonyl fluoride/40 mM NaCl). The homogenate was centrifuged at 105,000 xg for 60 min at 0°C in a Beckman L5-65 ultracentrifuge (Type 40.3 rotor). The clear cytosol was removed and used for receptor assay.

Glucocorticoid binding assay. In a preliminary experiment, 50 µl of above cytosol was incubated with different concentrations of [^{3}H] dexamethasone at 0°C to determine the maximum saturable concentration and timing for binding to receptors. Saturation of specific binding occurred after 2hr at 1.5-2.0X 10^{-8} M [^{3}H] dexamethasone in all brain regions without further change in binding. 50 µl of clear cytosols from each brain region were incubated at 0°C for 2h with 2X10^{-8} M[^{3}H]dexamethasone alone or with 2X10^{-5} M nonradioactive dexamethasone. Each assay was done in quadruplicate.

Following incubation, the entire reaction mixture was spotted onto dry 2.4 cm glass microfibre filter (GF/A) (13). After 10 min of incubation at room temperature, filters were washed three times (15 min each) in 20 ml of NET buffer (10 mM Tris-HCl, pH 8.1/1mM Na_2EDTA/40 mM NaCl) per filter at 0-4°C with continuous shaking. Excess liquid was removed from each filter by keeping briefly under heat lamp on aluminum foil. Radioactivity in the dried filters was counted in a complete counting cocktail (3a70B) using Beckman LS-100C liquid scintillation counter with efficiency of 51.1% for tritium. The filter assay takes advantage of the strong affinity for the glucocorticoid receptors to the glass fibre filter. The background of free [^{3}H] dexamethasone binding to the filters is approximately 0.05% of the added radioactivity. The protein content of the cytosols was determined (14) using bovine serum albumin as standard. Receptor concentration was expressed as fmol/mg protein. Specific saturable binding was calculated by subtracting the radioactivity bound in the presence of a 1000-fold excess of the unlabelled dexamethasone from that bound in the presence of the labelled hormone alone. The data were statistically analyzed (15).

RESULTS AND DISCUSSION

Altered responsiveness to certain hormonal and other biochemical stimuli are age-associated phenomena (2). Glucocorticoids are involved not only in cellular growth and differentiation but also in the metabolic functions of various tissues of animals (16). It is evident that response to stress also depends on the age and is mediated through glucocorticoids which act on neural tissues by a common mechanism i.e. binding to intracellular receptors followed by transcriptional modulation of protein synthesis (1,19). In the present study a synthetic glucocorticoid, dexamethasone, was used for receptor assay because it possesses purer glucocorticoid properties than corticosterone and hydrocortisone as determined by their physiological actions in peripheral tissues and its very little interaction with plasma transcortin. Our preliminary observations show that the specific saturable binding of [^{3}H] dexamethasone was maximum at the concentration of 2 X 10^{-8}M for 2h. This is similar to the earlier reports (6,12,17). Our results indicate that maximum saturable binding (fmol/mg protein) for dexamethasone show a significant regional difference with the highest level in the hippocampus and lowest in the hypothalamus in the adrenalectomized rats at both ages. This differential distribution is consistent with earlier findings that corticosterone receptors are found in various brain areas with the highest concentration in the hippocampus (5,17,18). Our data (figs 1&2) show, also, lesser dexamethasone binding in intact than in adrenalectomized animals in all brain areas at both ages, with the most profound difference in the hippocampus. The fact that in intact animals the hippocampal

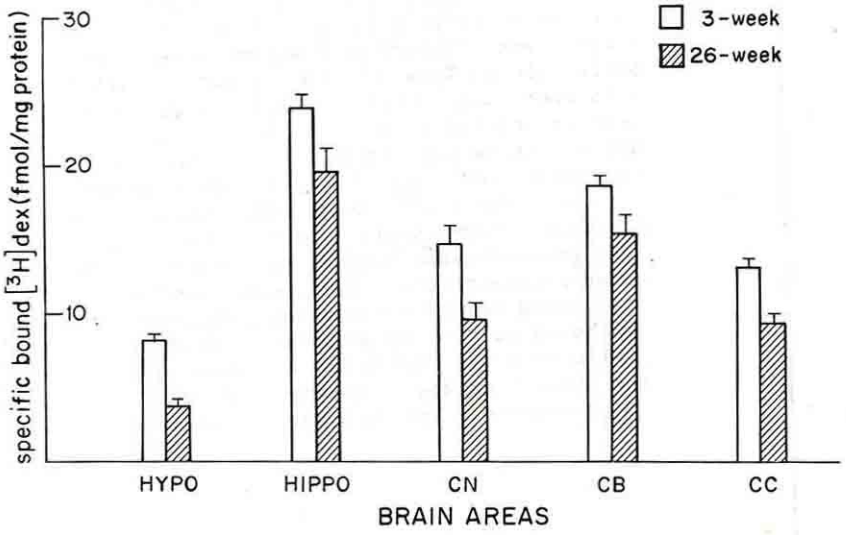

Figure 1. Saturable cytosolic specific binding of [^{3}H] dexamethasone (fmol/ mg protein) in hypothalamus(HYPO), hippocampus(HIPPO), caudate nucleus(CN), cerebellum(CB) and cerebral cortex(CC) of 3- and 26-week old adrenalectomized Long-Evans male rats. Results are mean $\pm$ S.D. of the 4-5 samples collected from 4-5 rats of each age group.

binding sites are much lower than in adrenalectomized suggests higher occupancy due to the presence of endogenous hormone. Our findings support the view of the hippocampus as a mediator of glucocorticoid effects upon the brain. Furthermore, in intact animals, regional differences at both ages are less evident than in adrenalectomized. Interestingly, this differential regional occupancy of endogenous glucocorticoid to receptor protein is also an age-associated phenomenon. It has been reported (6) that the amount of [^{3}H] corticosterone bound/mg protein to brain cytosol rapidly increases during the first 24 hr after adrenalectomy and approaches peak value at 3 days.

Our results (fig. 1) show that the endogenous levels of specific dexamethasone binding are higher in almost all brain regions of adrenalectomized immature (3-week) rats as compared to fully mature (26 week) rats. The higher level of glucocorticoid receptors in immature brain regions may be a contributory factor for more maturational events during the early phase of the life-span (3,19). We have observed a significant decrease (20-35%) in [^{3}H] dexamethasone binding in almost all regions of the brain of 26 week rats. This may reflect the gradual senescence of the

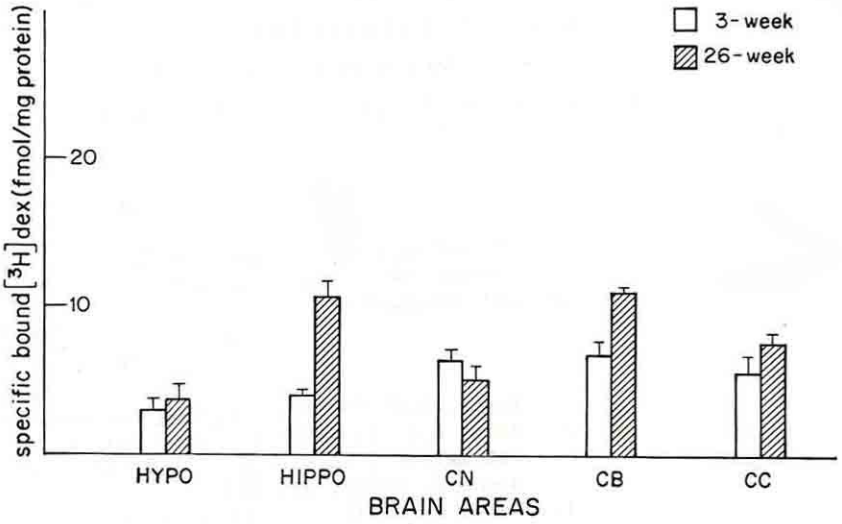

Figure 2. Saturable cytosolic specific binding of [^{3}H] dexamethasone (fmol/ mg protein) in hypothalamus(HYPO), hippocampus(HIPPO), caudate nucleus(CN), cerebellum(CB) and cerebral cortex(CC) of 3- and 26-week old normal intact male rats. Results are abbreviated as in figure 1.

adaptive mechanism(s). Using adrenalectomized rats, it has been reported that the brain cytosol receptor level increases to peak value in around 15-19 days after birth (3). In addition, we report here a moderate but statistically significant decrease in the dexamethasone receptors in all the five brain regions of the adrenalectomized rats of 26 weeks of age. The decrease is more pronounced in hypothalamus as compared to other regions. Stevens et al. (6) also reported a significant increase in corticosterone binding proteins in whole brain up to the age of 15-19 days. They correlated the findings with developing stress response which reaches nearly normal levels by day 21. Roth has reported a decrease in the amount of glucocorticoid bound in brain cytosol of old rats when 2- and 25-month old animals were compared (4). From our results, it is obvious that the decrease in receptor level starts not in old age but after reaching reproductive maturity. Sharma and Patnaik (20-22) reported that the magnitude of induction of brain cytosolic maleate dehydrogenase, aspartate aminotransferase, and phosphoenolpyruvate carboxyklinase by hydrocortisone is maximum at the age of 6 weeks and decreases by 20-30% at the age of 30 weeks, and further in older rats. Our present findings support the above observations that receptor levels for glucocorticoids are also decreasing in

the brain. It is concluded that glucocorticoid receptors show a differential distribution in different regions of the rat brain and their level is very much dependent on the physiological and biochemical changes which are occurring in different phases of the life span.

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