Expression of Glucocorticoid Receptor in the Brain of Rats during Postnatal Ontogeny

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 8, pp. 139-143, August, 2008
Original article submitted December 18, 2007

Expression of glucocorticoid receptor protein in the hippocampus and frontal cortex of male and female rats during the 1st, 2nd, and 3rd weeks of life was studied by Western blot hybridization. In the frontal cortex, the concentration of receptor protein progressively increased from the 1st to the 3rd week of life; in females, expression of 94-kDa protein significantly surpassed that in males during the 1st week of life. In the hippocampus, expression of 94-kDa and 82-kDa proteins during the 1st week of life was higher in males. Moreover, expression of the major glucocorticoid receptor isoform (94 kDa) in this structure remained unchanged in all periods of the study in males, whereas in females it was low over the first 2 weeks of life and increased by the 3rd week. Variations in the expression of glucocorticoid receptors in the hippocampus of male and female rats coincide with changes in plasma corticosterone concentration during the early postnatal ontogeny.

Key Words: *glucocorticoid receptors; brain; corticosterone; rat*

The maintenance of the balance between glucocorticoid hormones in the blood during the early ontogeny is the major factor for the growth and differentiation of various systems in the organism (primarily of the central nervous system) [1,2,10]. Increased level of glucocorticoids during the early postnatal ontogeny modulates the major processes of brain maturation, which results in programming of neuroendocrine function in adult animals [3,4,12].

The programming role of glucocorticoids during the early ontogeny manifests in modulation of the hypothalamic—pituitary—adrenocortical system (HPAS). Dysfunction of HPAS in adult animals is induced by exogenous hormones. Moreover, stress exposure is followed by activation of HPAS during the first 2 weeks of life [7,8,11,12]. This period of life (days 2-14 of postnatal development) in altricial animals, including rats, received the name "areactive stage" [13]. Low activity of HPAS manifests in reduced concentration of corticotropin-releasing hormone in the hypothalamus and ACTH in adenohypophysis, extremely low plasma corticosterone concentration, and the absence of stress activation of the adrenal glands in response to many, but not all stress stimuli [11,15]. Reactivity of the adrenal glands in stress stimulation is manifested on day 14 of postnatal ontogeny and becomes mature during the pubertal period [14].

The hippocampus and frontal cortex are involved in the regulation of HPAS in adult animals. Glucocorticoid receptors (GR) in these structures contribute to induction and inhibition (negative feedback regulation) of stress activity of HPAS [6]. The role of brain structures in the maintenance of HPAS function during the early postnatal ontogeny remains unknown.

Our previous studies showed that hippocampal GR in male rats are presented by several proteins with different molecular weight [5]. These proteins were characterized by certain pattern of expression

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0007-4888/08/1462-0176

during all periods of postnatal ontogeny. The last day of the "areactive period" was a critical moment for variations in the ratio between various forms of hippocampal GR. Hence, it was interesting to study the expression of GR in the frontal cortex of males and the existence of similar regulations in females during the early postnatal ontogeny. Basal activity of HPAS was estimated from plasma corticosterone concentration in males and females of different age.

MATERIALS AND METHODS

Experiments were performed on Sprague-Dawley male and female rats. Pregnant rats were placed in individual cages and examined daily to determine the date of labor. The day of appearance of newborn rat pups was considered as day 0 of life. On the next day, the number of rat pups in litters was brought to 8 (4 males and 4 females). The litters with predominance of males or females were excluded from further observations. A total of 18 litters were examined. Female rats and litters were maintained in a vivarium under standard conditions and had free access to water and food. The study was conducted according to ethics rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EEC). The scheme of experiments was approved by Committee on Animal Humanity (I. P. Pavlov Institute of Physiology).

Rat pups were rapidly isolated from home cages and decapitated on days 3, 5, 10, 15, 18, 21, and 30 of postnatal ontogeny. The blood was collected during decapitation and centrifuged at 1000*g* and 4°C for 20 min. The plasma was stored at -20°C until corticosterone assay. Plasma corticosterone concentration was measured by radioimmunoassay with antiserum and [1,2,6,7-^{3}H]-corticosterone (specific activity 76.5 Ci/mmol, NEM™ Life Science Products) [12].

Brain samples from rat pups (days 5, 15, and 21 of life) were obtained on ice. The frontal cortex and hippocampus were isolated and homogenized. The expression of GR protein was measured by Western blot hybridization [5]. Brain tissues were homogenized with lysing buffer containing proteinase inhibitors. The homogenates were centrifuged at 32,000g and 4°C for 1 h, the supernatants were stored at -70°C for not more than 1 month.

Total protein concentration in samples was measured using BCA Protein Assay Kit (Pierce, Rockford, IL). Individual samples with the same protein content (25 μg) were mixed with Laemmli buffer. Protein was denatured by heating at 95°C for 5 min. The samples were layered on 7.5% polyacrylamide gel and fractionated by electrophoresis. After separation in gel, the proteins were electrophoretically transferred to nitrocellulose membrane using a transfer buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol (pH 8.3). The membranes were incubated in a blocking solution of 5% milk with TBST buffer (50 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20; pH 8.0) at 37°C for 1 h. The membranes were put in 1% milk solution in TBST buffer containing polyclonal antibodies against GR (GR (M-20), Santa Cruz Biotechnology Inc.) or monoclonal antibodies against β-actin (AC-15, Sigma-Aldrich laboratories) in the 1:500 dilution. After overnight incubation at 4°C, the membranes were washed 3 times with TBST buffer (10 min) and incubated in the presence of 1% milk solution in TBST buffer with horseradish peroxidase-conjugated secondary antibodies (dilution 1:2500) at room temperature and constant agitation for 1 h. Proteins were visualized with detection reagents (ECL detection kit, Sigma-Aldrich laboratories) according to manufacturer's recommendations. Optical density of GR protein bands was measured using Image ProPlus software (Media Cybernetics Inc.).

The results were analyzed by Mann—Whitney *U* test. The differences were significant at $p<0.05$.

RESULTS

Corticosterone concentration significantly differed in males and females (Fig. 1). The opposite differences in corticosterone concentration were observed at all stages of postnatal ontogeny. Until day 15 of life, blood corticosterone concentration in males was much higher than in females; this parameter progressively increased by day 30 of life. In

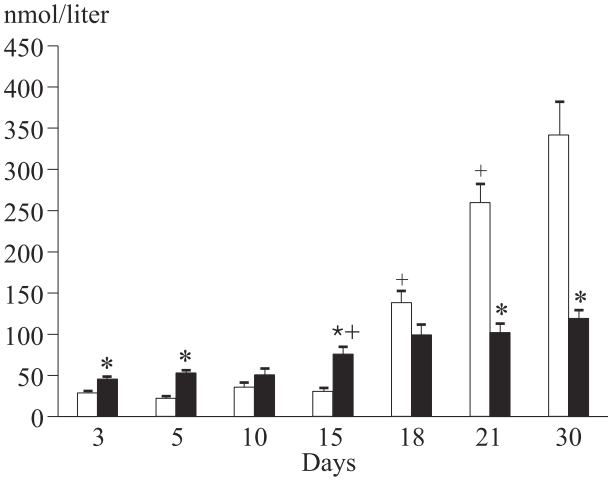

Fig. 1. Basal corticosterone concentration in blood plasma from male and female rats during early postnatal ontogeny. Light bars, females; dark bars, males. $p<0.05$: *compared to females; +compared to previous period of life.

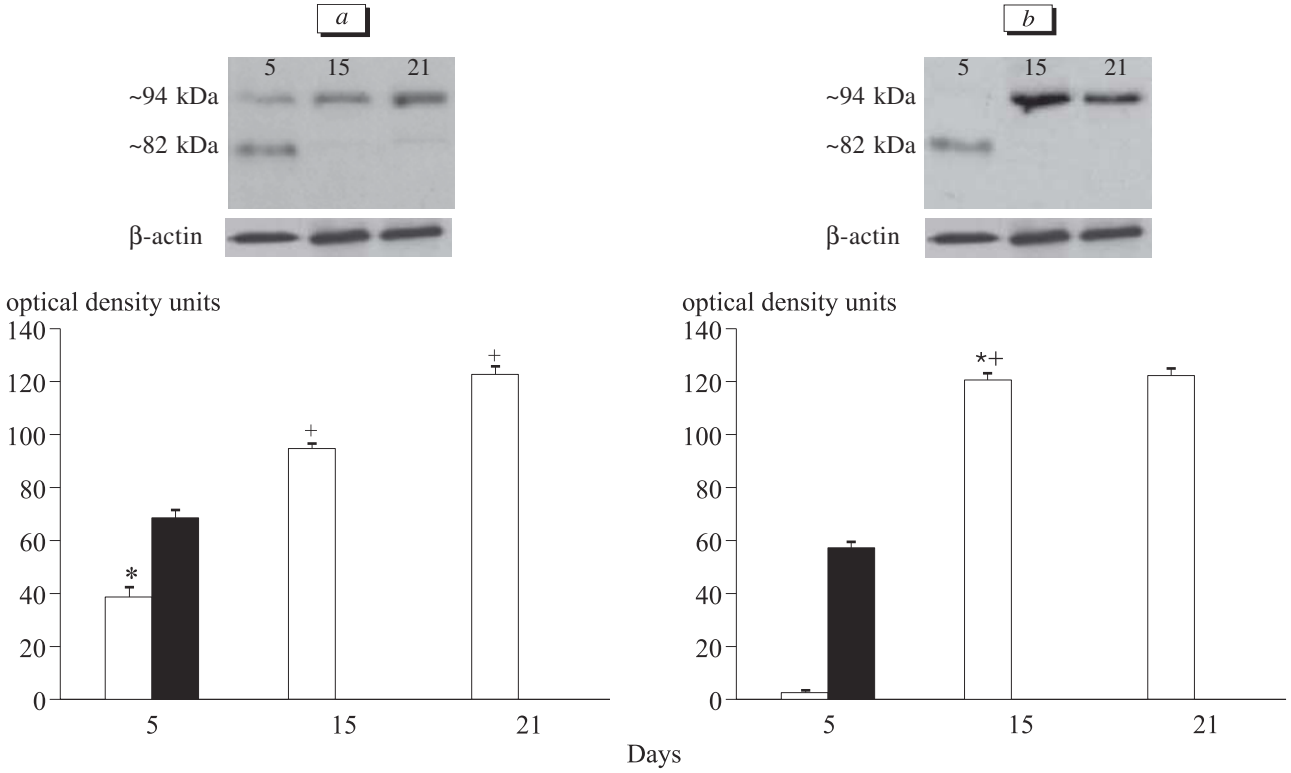

Fig. 2. Ontogenetic pattern of GR expression in the frontal cortex of rats during postnatal ontogeny. Here and in Fig. 3: (*a*) females; (*b*) males. Light bars, 94-kDa GR; dark bars, 82-kDa GR. $p<0.05$: *significant gender differences; +compared to previous period of life.

females, corticosterone concentration remained low on day 15 of life, but sharply increased starting from day 18 and surpassed that in males.

Considerable gender differences in the expression of GR protein were found on the 1st, 2nd, and 3rd weeks of life. GR proteins of 94 and 82 kDa were detected in the frontal cortex of females on day 5 of life (Fig. 2, *a*), whereas in 5-day-old males expression of 94-kDa GR was extremely low, but this parameter significantly increased in the follow-up period and surpassed that in females (at least on day 15 of life; Fig. 2, *b*). In females, expression of 94-kDa protein also increased from the 1st to the 3rd week of life, but this increase was less pronounced than in males.

Gender differences in the expression of GR protein were most pronounced in rat hippocampus. For example, expression of GR proteins with molecular weights of 94 and 82 kDa in 5-day-old females was much lower than in males. The expression of these proteins in females remained low on day 15, but significantly increased by the 3rd week of life (Fig. 3, *a*). The expression of 94-kDa protein in the hippocampus of males remained practically unchanged in all periods of life (Fig. 3, *b*).

Our previous studies showed that hippocampal GR in male rats exist in the forms with different molecular weights [5]. They correspond to various isoforms of GR-A, GR-S, and GR-D [9]. These proteins are characterized by a certain pattern of expression during postnatal ontogeny. GR-A (94 kDa) was detected in all periods of postnatal ontogeny. GR-S (82 kDa) disappeared on day 13 of life, but was present in adult animals. GR-D expression (54 kDa) was first revealed on day 13 of life and increased in the follow-up period [5].

Here, we studied the expression of two major isoforms of GR (A and S). These isoforms are characterized by similar expression of GR not only in the hippocampus, but also in the frontal cortex of males. Similar results were obtained for the hippocampus and frontal cortex of females. We revealed the existence of regional and gender differences in the expression of GR isoforms. It should be emphasized that over the first 2 weeks of life, the expression of the major isoform GR-A in the hippocampus of females was much lower than in males. This parameter increased by the 3rd week of life, which corresponds to variations in plasma corticosterone concentration during early postnatal ontogeny. GR-A expression in the frontal cortex of females and males increased from the 1st to the 2nd week of life. However, the increase in GR-A expression in males was more significant than in females.

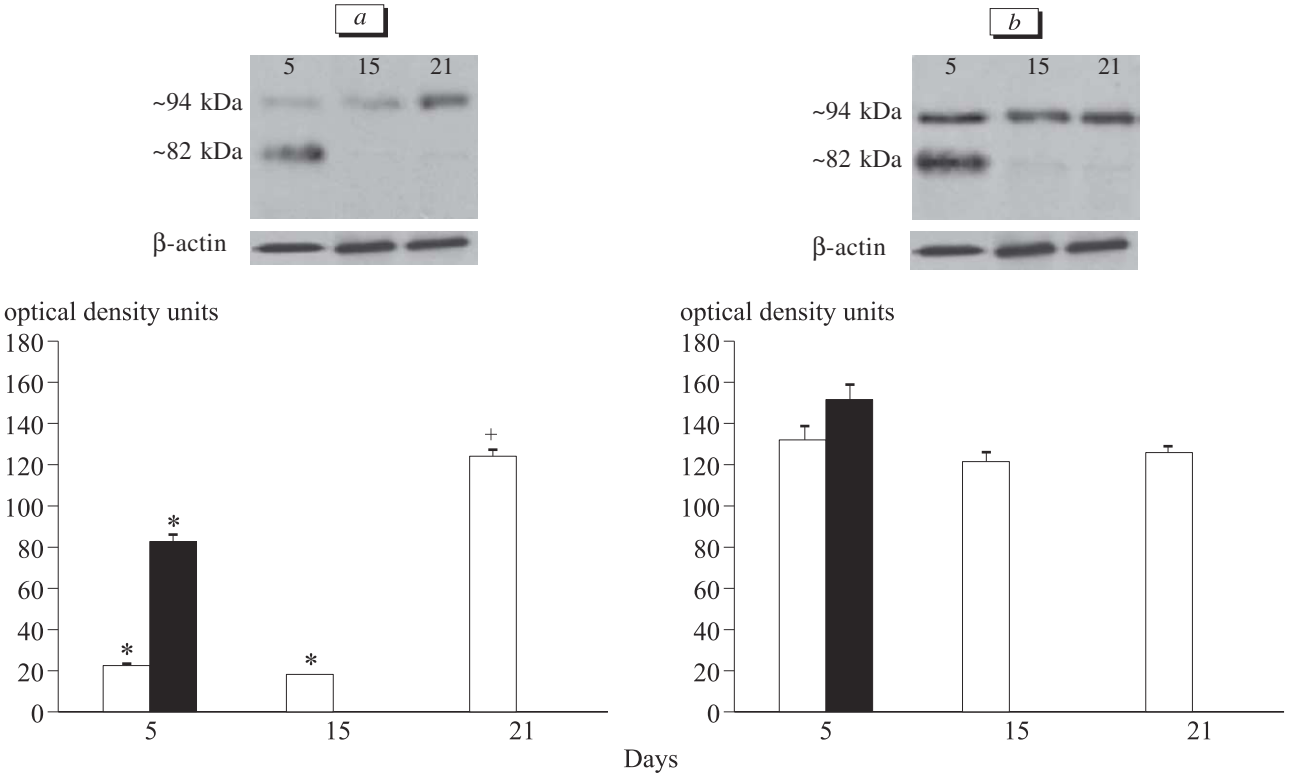

Fig. 3. Dynamics of GR expression in the hippocampus of rats.

Studying the expression of GR in the brain of male and female rats during postnatal ontogeny revealed the existence of significant gender differences in the expression of the major isoforms GR-A and GR-S in the frontal cortex and hippocampus. The study of variations in blood corticosterone concentration showed that hippocampal GR-A are involved in the maintenance of low basal activity of HPAS over the first 2 weeks of life.

This work was supported by the St. Petersburg Scientific Center of the Russian Academy of Sciences (grant No. 113IF, 2007) and grant of the President of the Russian Federation (NSh-1434.2008.4).

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