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BRAIN CORTICOSTEROID RECEPTOR GENE EXPRESSION AND NEUROENDOCRINE DYNAMICS DURING AGING

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Summary—The present study examined the stress responsiveness of the hypothalamic–pituitary–adrenal axis in relation to the properties of corticosteroid receptors in the brain and pituitary in old (30 months) and young (3 months) male Brown Norway rats. The data demonstrate that circulating ACTH rather than the corticosteroid plasma level was elevated under basal conditions and following stress. Furthermore, a reduction of mineralocorticoid receptor (MR) number in the hippocampus and of glucocorticoid receptor (GR) number in the hypothalamus and the pituitary correspond to increased neuroendocrine responsiveness and negative feedback following stress. The changes in receptor binding do not parallel the changes in the amount of MR and GR mRNA measured with *in situ* hybridization. This suggests that the processing rather than the receptor gene expression is affected in senescence.

INTRODUCTION

Corticosteroids are believed to be of critical importance in the control of homeostasis. Their action in the brain is mediated by two types of corticosteroid receptors: the mineralocorticoid (MR) and the glucocorticoid receptor (GR). Differentiation between MR and GR is based on their primary structure, localization, regulation and function [1–3]. MR is predominantly expressed in the hippocampus [4–6], and *in vivo* preferentially binds corticosterone (B) in the rat [7]. In contrast, GR is widely expressed in neurons and glial cells and displays a 6 to 10-fold lower affinity for B [6, 8].

Recent neuroendocrine studies revealed that both MR and GR are involved in the effect of B on the stress responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis [1]. The limbic MR-mediated effects of B are involved in basal control of HPA activity [9, 10], whereas GR-mediated effects in the hypothalamus and pituitary block stress or circadian-induced activity of the HPA axis [1, 9, 11].

The aging process is characterized by an impaired corticosteroid receptor regulation [12–15], an altered activity of the HPA axis under resting as well as stress conditions [14, 16, 17] and a decreased ability to adapt and to maintain homeostasis [18]. We have examined the relation between changes in MR properties relative to those of GR and changes in HPA reactivity to stress in the male Brown Norway rat during senescence.

EXPERIMENTAL

Young (3 months) and old (30 months, IVEG/TNO, Rijswijk, The Netherlands) male Brown Norway rats were housed individually under standard lighting and temperature conditions with food and water *ad libitum*. The rats were handled daily starting a week prior to surgery. Silicone cannulas (i.e. 0.5 mm, o.d. 1.0 mm) were implanted into the entrance to the right atrium via the external jugular vein according to the method of Steffens *et al.* [19]. Surgery was performed under fentanyl anesthesia (0.02 mg/100 μl/150 g body wt i.m.) and under sterile conditions. During the 1-week recovery period, the animals were handled daily and their cannulas were checked, which permitted habituation to the blood sampling procedure [10]. The rats predestinated for stress were challenged with a conditioned emotional response (slight modification of the procedure described previously [20]). Six blood samples were taken over a period of 2 h following stress as well as in the non-stressed condition. Blood samples of 300 μl were withdrawn with sterile

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heparinized syringes and processed for hormone determination. The loss of body fluid was compensated by immediate i.v. injection of 300 μl of sterile saline. On days 12, 13 and 14 of the handling procedure prior to experimentation, morning blood samples collected during the daily cannula check were used for assessment of basal plasma levels of B.

Plasma B was measured by RIA using an antiserum raised in sheep against corticosterone-21-hemisuccinate bovine serum albumin (gift of Dr Th. Benraad, University of Nijmegen, The Netherlands). The method used is described elsewhere [21, 22]. Plasma ACTH was measured by RIA using a commercially available antiserum raised in rabbit and directed against the amino acid sequence 5–18 of the ACTH molecule (IgG Corporation, Nashville, TN, U.S.A.), as described previously [23].

Brain and pituitary MR and GR and plasma CBG binding constants were determined in 24 h adrenalectomized young and aged rats. The *in vitro* cytosol assays were carried out according to the previously established method [7], with slight modifications. The wet weight of the thymus and adrenal glands from these animals was determined.

The *in situ* hybridization procedure to detect MR and GR mRNA has been described elsewhere [6]. The antisense probe was transcribed from a 513 basepair (bp) rat brain MR cDNA fragment (gift of J. L. Arriza, La Jolla, CA, U.S.A.) and a 500 bp rat liver GR cDNA fragment (gift of M. C. Bohn, University of Rochester, NY, U.S.A.). The autoradiographic images obtained were quantitated with a computer-assisted image analysis system ($n = 9$ to 12 sections/3 brains/probe).

Basic locomotor behavior was tested with the equilibrium test on a small and broad bar, with the equilibrium test on a tilted platform and the placing reaction test on the edge of a surface.

The results are expressed as mean $\pm$ SEM. Statistical analysis of hormone plasma levels was performed with multivariant MANOVA analysis using Wilks lambda. In all other cases, a paired Student's t-test was applied.

RESULTS

Corticosterone (B)

Basal plasma B levels were not significantly different between aged and young rats [4.7 ± 0.3 μg/dl ($n = 15$) vs 5.0 ± 0.3 μg/dl ($n = 18$), respectively]. B secretion increased in response to stress to maximum levels of 17.5 ± 1.2 μg/dl (old rats) and 14.7 ± 2.3 μg/dl (young rats) at $t = 20$ and 30 [Fig. 1(A)]. In aged rats a tendency to secrete higher amounts of B within the first 60 min of the experiment was observed. Statistical analysis, however, did not reveal significant differences. In the non-stressed condition [Fig. 1(B)], no differences were found between the two ages.

ACTH

Aged as opposed to young rats displayed higher basal plasma levels of ACTH when determined at $t = 0$ [45.2 ± 1.7 ($n = 7$) vs 37.2 ± 1.7 pg/ml ($n = 8$), respectively; $P < 0.05$]. Exposure to stress resulted in a significantly higher elevation of ACTH in the aged rat [peak level old rats = 182.7 ± 11.3 pg/ml, peak level young rats = 90.4 ± 24 pg/ml at $t = 20$; Fig. 1(C)]. The average release of ACTH during the 2 h testing period was higher in the aged than the young rats ($P < 0.001$). In addition, a more prolonged elevation of the plasma ACTH following the conditioned emotional response test was found in the aged rats ($P < 0.05$). In the non-stressed condition, aged rats showed higher concentrations of plasma ACTH during the entire period of testing [$P < 0.05$; Fig. 1(D)].

Corticosteroid receptors

Maximal binding capacities (B_{max}) of MR and GR in the hippocampus, hypothalamus and pituitary are presented in Table 1. MR revealed a decreased binding capacity in the hippocampus at older age (−44%). No age-related change was observed in the number of hippocampal GR binding sites. However, the B_{max} of GR was decreased in the hypothalamus (−40%) and the anterior pituitary (−50%).

The distribution pattern of MR and GR mRNA in the hippocampus and hypothalamus appeared similar in young and aged rats [6]. Quantitative image analysis of [^{35}S]MR and GR mRNA autoradiograms revealed that GR mRNA was significantly reduced in pyramidal cell field CA_3 ($P < 0.001$; −42%), CA_4 ($P < 0.01$; −41%) and the dentate gyrus ($P < 0.05$; −26%) of the hippocampus in aged rats. No change was found in the hypothalamic PVN. MR mRNA levels in all hippocampal cell fields of the aged rat were not significantly different from the young rat.

The adrenals of aged rats were significantly increased [$P < 0.001$; 73.0 ± 2.6 mg ($n = 31$ old rats) vs 48.5 ± 1.7 ($n = 30$ young rats)], whereas

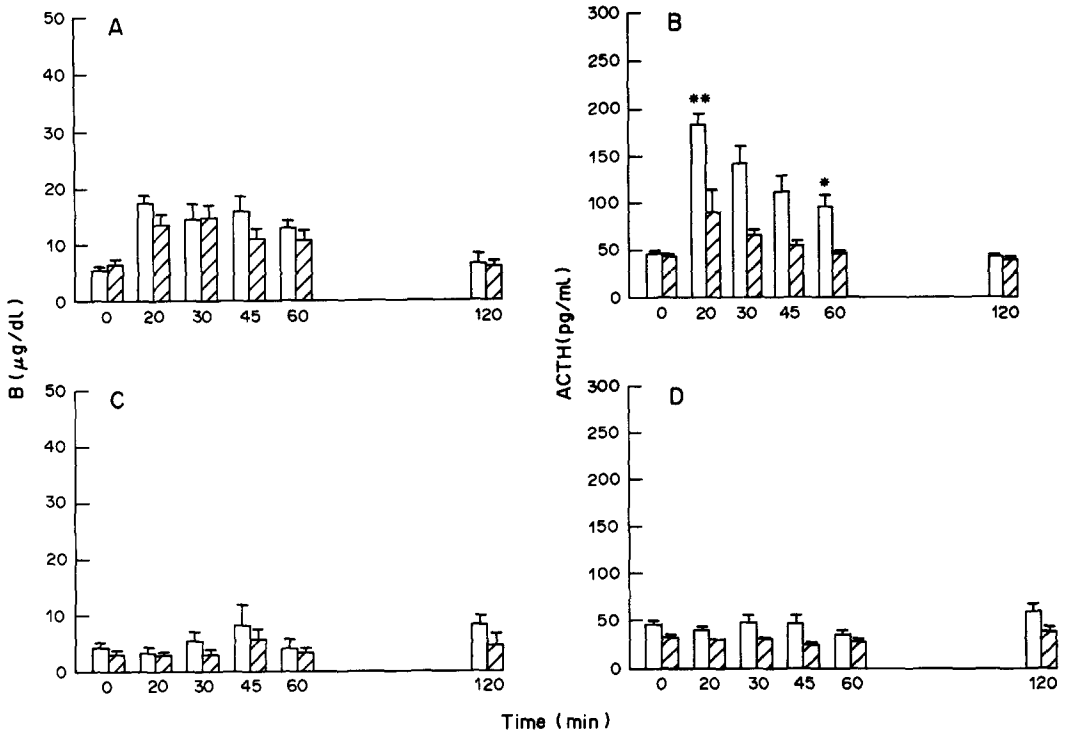

Fig. 1. Effect of the conditioned emotional response on plasma B levels (A, μg/dl) and ACTH (B, pg/ml) levels in young (▨; $n = 8$) and aged (□; $n = 6$) Brown Norway rats. Plasma B (C) and ACTH (D) levels in the paired control procedure are presented (young, $n = 5$; old, $n = 7$). The results are expressed as mean ± SEM time effect $P < 0.05$, group effect $P < 0.001$, time interaction effect $P < 0.05$ ($^*P < 0.01$, $^{**}P < 0.001$).

thymus wet weight was significantly less in aged rats [$P < 0.001$; 72.7 ± 7 mg [$n = 16$ old rats] vs 256,2 ± 9 mg ($n = 23$ young rats)]. Basic locomotor abilities were markedly reduced in aged rats, especially motoric behavior of the hind legs.

DISCUSSION

The present study demonstrates that ACTH release from the pituitary is a more sensitive indicator of age-induced changes in HPA activity compared to adrenocortical secretion. Aging increased and prolonged ACTH release in response to the conditioned emotional response test, whereas only a trend towards a more prolonged elevation of plasma B could be measured. In addition, we measured higher resting levels of ACTH in the old male Brown Norway rats as opposed to unaltered basal plasma B levels.

Table 1. B_{max} of MR and GR binding sites in the Brown Norway rat

	MR		GR	
	Young	Old	Young	Old
Hippocampus	390.8	217.7	196.3	198.0
Hypothalamus	ND	ND	395.7	235.7
Ant. pituitary	ND	ND	295.0	148.6

n = number of animals for pooled tissue; young ($n = 10$), old ($n = 7$). ND = not detectable above non-specific; data are expressed in fmol/mg protein.

A number of studies have examined the impact of aging on HPA activity. The resulting data, however, do not always agree. Some studies report higher basal levels of circulating B [24] and ACTH [26] during aging, whereas others found plasma B levels to be unchanged [14, 25, 26]. A more prolonged elevation of stress-induced plasma B has been reported [27], whereas attenuated termination of stress-induced ACTH release was only found following chronic stress [25]. This inconsistency may reflect the occurrence of large individual differences in stress responsiveness among the group of aged rats. Alternatively, this could be explained by the use of a variety of different strains of animals ranging in age between 17.5–30 months and different challenges ranging in severity.

The present data are consistent with the view that aging induces greater reactivity of the HPA axis following stress [14]. In our observation, the age effect is amplified at the level of the pituitary and higher basal as well as stress and CRH-induced ACTH levels are observed. These data suggest that the pituitary is more sensitive to

ACTH secretagogues during senescence. Alternatively, the pituitary may be altered in sensitivity to GR-mediated corticosteroid feedback. Finally, also central receptor deficits can not be excluded in the aged animals.

We found that 24 h after adrenalectomy the aged rats displayed a reduced binding capacity of MR but not GR in the hippocampus. However, less GR binding was observed in the hypothalamus and anterior pituitary of aged rats. Quantitative *in situ* hybridization of cRNA probes to brain tissue corticosteroid receptor mRNA showed an effect of aging on the GR gene expression in the hippocampus. GR mRNA was decreased in cell field CA_3, CA_4 and the dentate gyrus. No such change could be measured in the hypothalamic PVN. MR mRNA levels in the hippocampus were not affected by age. Thus, age-related changes in receptor numbers and transcript abundancy do not always parallel. In parallel with the present study, however, a number of other studies show that the regulation of MR and GR is altered during aging [12–15]. Generally, a reduced hippocampal MR concentration is observed and GR is found reduced or impaired in other binding site properties. Accordingly, MR and GR plasticity seems reduced during senescence.

In order to evaluate a relationship between reductions in MR and GR in the brain and pituitary, and altered HPA activity during aging, central corticosteroid receptor heterogeneity and their distinct roles in neuroendocrine regulation have to be considered. Recent studies [9–11] have found central MR mediated effects to be functionally involved in a tonic suppression of basal HPA activity and responsiveness to stress, hypothalamic GR mediated effects to be involved in feedback action on stress-induced HPA activity, whereas hippocampal GR was found mostly involved in regulation of stress-induced behavior [11]. The glucocorticoid cascade hypothesis of Sapolsky *et al.* [17] suggests that chronically elevated plasma B levels compromise the viability of brain cells and to make these cells more vulnerable during aging. As a consequence, corticosteroid receptors in the hippocampus are reduced and glucocorticoid feedback is impaired which will leave long-term hypersecretion of B uncontrolled. However, P. Landfield proposes that age-induced changes in GR and GR-mediated feedback functions are adaptive responses aimed to protect the brain against the deleterious effects of corticosteroids during aging [12, 13, 28].

The present study shows data that are consistent with the view that reduced MR in the hippocampus in combination with reduced GR in the anterior pituitary and hypothalamic PVN account for increased basal and stress or CRH-induced plasma ACTH. We suggest that the role of corticosterone in the aging process should be considered in the context of MR as well as GR mediated action.

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