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LIMBIC CONTROL OF ENDOCRINE GLANDS IN AGED RATS

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Abstract—Two weeks after bilateral lesions in the dorsal and ventral hippocampus, septum, and basolateral amygdala, plasma concentrations of ACTH, TSH, corticosterone, aldosterone, and thyroxine were determined in 5–6-month-old (adult) and 24–26-month-old (aged) male Wistar rats. The results indicate that with age, limbic system control over the hypothalamic–pituitary–adrenal/thyroid axes changes, although these changes are not uniform. Thus, the inhibitory influences of the dorsal/ventral hippocampus and septum on ACTH release tend to diminish as age increases. However, the inhibitory influence of the amygdala on ACTH appears to become enhanced during the aging process. The data also support an enhanced sensitivity of the adrenal cortex to ACTH during aging. In adult animals, lesions of the dorsal/ventral hippocampus and amygdala result in significantly decreased plasma concentrations of TSH consistent with a stimulatory influence of these regions on TSH release. Lesions in the septum, however, lead to significantly increased TSH secretion in these animals. To these findings, lesions of the dorsal/ventral hippocampus and amygdala in aged animals resulted in significantly increased TSH secretion, while results of septal lesions were not significant.

Key Words: limbic system, aging, hippocampus, septum, amygdala, ACTH, TSH, corticosterone, aldosterone, thyroxine

INTRODUCTION

THE LIMBIC system plays an important role in the neural control of the hypothalamus (Oniani, 1983; Kling *et al.*, 1987). However, data on the influence of the limbic system on hormone secretion (e.g., corticosteroids and thyroid hormones) are controversial. Although some data support stimulatory effects of the limbic system on hormone secretion (Matheson *et al.*, 1971; Redgate and Fahringer, 1973; Dunn, 1987), other findings indicate inhibitory influences (Knigge, 1961; Dupont *et al.*, 1972; Dunn, 1987). These discrepancies may be due to differences in the initial level of hormone in the blood (especially in the case of glucocorticoids) (Nessen, 1981) or in the limbic system area used for analysis. It should be emphasized that there are no reported data on the effects of aging on the influence of limbic structures (hippocampus, amygdala, septum) on the secretion of the hormones mentioned previously.

However, during aging, significant morphological and functional changes have been observed in limbic structures, namely a degeneration of pyramidal cells in the hippo-

campus of humans (Scheibel and Scheibel, 1975) and rats (Barnes, 1979), accumulation of lipofuscin (Brizzee and Ordy, 1979), a decrease in the excitability of amygdala medial nuclei and an increased excitability of its central nuclei (Frolkis and Benrukov, 1971), and a deterioration of their vascularization. All of these changes may alter the influence of the limbic system on endocrine gland function.

The aim of this study was to investigate the influence of the dorsal and ventral hippocampus, septum, and amygdala on the secretion of corticosteroids and thyroid hormone during aging. Evaluation of limbic system influences on the hypothalamic control of corticosteroids and thyroid hormones would ideally involve evaluation of the secretion of hypothalamic corticotropin releasing hormone and thyrotropin releasing hormone. For the organism, the most important physiological implication is the final result of these influences. Hence, in the present study, the physiological consequences of changes in releasing hormone secretion were evaluated by measurement of the pituitary trophic hormones [i.e., adrenocorticotropin (ACTH) and thyrotropin (TSH)] as well as products of their target glands, corticosterone, and thyroxine. In addition, plasma levels of aldosterone, an adrenal mineralocorticoid, were determined.

MATERIALS AND METHODS

Male Wistar rats, ranging in age from 5–6 and 24–26 months old were used in this study. Limbic structure lesions were performed by electrocoagulation (DC 3 mA, 20 s) using monopolar nichrome electrodes with a free tip diameter of 0.15 mm. The electrodes were inserted into the corresponding brain structures of anesthetized (pentobarbital 40 mg/kg, IP) animals according to stereotaxic atlas coordinates (Fifkova and Marsala, 1967). In each animal, one of the structures of the limbic system was bilaterally lesioned. Coordinates for electrode insertion (corrected for age) were as follows: dorsal hippocampus, adult rats: A-P −3 mm, Lat 2 mm, D-V 4 mm; old rats: A-P −3 mm, Lat 2 mm, D-V 4.5 mm; ventral hippocampus, adult rats: A-P −4.5 mm, Lat 4.5 mm, D-V 8.5 mm; old rats: A-P −4.6 mm, Lat 4.9 mm, D-V 8.9 mm; septum adult rats: A-P +1 mm, Lat 0.3 mm, D-V 5.5 mm, old rats: A-P +1 mm, Lat 0.3 mm, D-V 6 mm; basolateral amygdala, adult rats: A-P −2 mm, Lat 3.7 mm, D-V 9.9 mm, old rats: A-P −2 mm, Lat 4 mm, D-V 10.3 mm. After electrocoagulation, the electrodes were removed and the skull wounds sutured. Following sacrifice of animals, the locations of the lesions were verified by comparing brain semithin (120 μ) sections (freezing microtome) with the stereotaxic atlas.

Prior to and 2 weeks following the lesions, tail blood samples were collected from anesthetized animals. Hormone concentrations were determined by radioimmunoassay (RIA). The following kits were employed: ACTH-AKTHK-IPR (ORISIndustrie, France); TSH (Byk-Sangtec Diagnostica, Germany); corticosterone—Institute of Experimental Pathology and Therapy (Sukhumi, Abkhasia); aldosterone-ALDOK (ORIS Industrie, France); and thyroxine-RIA-T_4-RH (Minsk, Byeloruss).

RESULTS AND DISCUSSION

ACTH and corticosteroids

Limbic structures play an important role in the control of corticosteroid secretion. Electrical stimulation of the hippocampus inhibits ACTH secretion (Mason *et al.*, 1961) and causes a decrease of plasma corticosteroids (Dupont *et al.*, 1972). Stimulation of

the dorsal hippocampus in the presence of a stress inducer inhibits the release of ACTH and corticosteroid secretion. Also, inhibition of the hypothalamic–pituitary–adrenal axis by the hippocampus was demonstrated in experiments where hippocampus lesions caused an increase in corticosteroid secretion (Knigge, 1961). However, Sapronov (1979) and Wilson and Critchlow (1973) did not find any changes in the pituitary–adrenal axis following hippocampectomy. A possible explanation of these contradictory data was provided by Nessen (1974), who demonstrated that the effects of hippocampal stimulation on the pituitary–adrenal axis was dependent on the initial level of corticosteroids in the blood. If the initial levels of corticosteroids were low, stimulation of the dorsal hippocampus had no effect; however, if the initial levels of corticosteroids were high, hippocampal stimulation inhibited secretion. In addition, stimulation of the ventral hippocampus at initially low levels of corticosteroids caused an increase in their secretion. The author concluded that the hippocampus could inhibit or stimulate hypothalamic function, depending on the intensity of the influences of negative corticosterone feedback. It should be emphasized that the highest concentration of glucocorticoid and mineralocorticoid receptors in the brain are found in the hippocampus. It is of interest to note that in rhesus monkeys, no changes in aldosterone secretion were observed following amygdala and hippocampal stimulation (Frankel *et al.*, 1976).

In adult rats 2 weeks after lesions of dorsal hippocampus the concentration of ACTH in blood plasma was increased by 419 ng/l. Accordingly, there were increases in the concentration of both corticosterone (470 nmol/l) and aldosterone (1045 pmol/l) (Table 1).

In old animals, the ACTH level increased by 62 ng/l only, and the concentration of corticosterone increased in a similar manner (479 nmol/l), as observed in adult rats. At the same time, aldosterone concentration in blood plasma of old animals did not change significantly.

TABLE 1. INFLUENCE OF LIMBIC STRUCTURE LESIONS ON ACTH AND CORTICOSTEROIDS CONCENTRATION IN PLASMA OF ADULT AND OLD RATS

Limbic structure	*Age group*	*ACTH (ng/l)*		*Corticosterone (nmol/l)*		*Aldosterone (pmol/l)*	
		Initial	*14 days*	*Initial*	*14 days*	*Initial*	*14 days*
Dorsal hippocampus	Adult	90.4 ± 1.9	509 ± 28*	298 ± 19	1769 ± 84*	430 ± 93	1475 ± 482†
	Old	93.7 ± 2.3	156 ± 6*	296 ± 14	776 ± 108†	943 ± 113	790 ± 221
			$p < 0.001$			$p < 0.05$	
Ventral hippocampus	Adult	55.3 ± 2.5	706 ± 18*	286 ± 7	1452 ± 24*	505 ± 82	1670 ± 306†
	Old	78.6 ± 4.3	82.4 ± 2.0	288.6 ± 10.5	274.9 ± 28.7	1123 ± 113	1262 ± 238
		$p < 0.01$	$p < 0.001$		$p < 0.001$	$p < 0.01$	
Septum	Adult	57.8 ± 2.6	810 ± 52*	296 ± 19	1013 ± 28*	527 ± 115	1804 ± 311†
	Old	86.7 ± 1.9	174 ± 10*	295 ± 13	934 ± 37*	569 ± 99	1532 ± 395†
		$p < 0.001$	$p < 0.001$				
Amygdala	Adult	52.7 ± 6.7	755 ± 25*	299 ± 10	1142 ± 32*	1156 ± 263	2511 ± 343†
	Old	97.9 ± 3.9	941 ± 23*	306 ± 6	1514 ± 25*	815 ± 102	772 ± 183
		$p < 0.001$	$p < 0.001$		$p < 0.001$		$p < 0.01$

*$p < 0.001$; †$p < 0.01$ = Significant changes in the same age group.
p = significant difference between age groups.

If one takes into account the fact that body weight and consequently blood volume in old rats are larger than in the younger adult, the same shift of the corticosterone concentration in blood plasma of both age groups was achieved in old rats due to more intensive secretion by their adrenals. The data obtained indicate a weakening of dorsal hippocampus influences on CRH secretion as well as an increase in the sensitivity of cells of zona fasciculata of adrenals to the ACTH influences in aging.

Two weeks after a ventral hippocampus lesion in adult animals, the same though more pronounced shifts of ACTH and corticosterone concentrations in blood plasma were registered: ACTH level was increased by 650 ng/l and corticosterone by 1165 ng/l. At the same time, in old rats with lesioned ventral hippocampi, there were no noted changes in the concentration of these hormones in comparison with the initial levels. These data also testify to the decrease of hippocampal control over the functional state of this system in regulating the intensity of corticosteroids secretion in old age.

Two weeks after electric coagulation of the septal area in adult rats a significant increase in the level of hormones studied was observed: ACTH concentration was increased by 753 ng/l, while the concentrations of corticosterone and aldosterone were increased 3.4 times.

In old animals with a septum lesion only, slight changes of ACTH concentration in blood plasma (87 ng/l) were observed, but corticosteroid concentration increased to the level of those observed in adult rats.

Whereas hippocampal lesions caused a more pronounced change in ACTH in blood plasma in adult animals as compared to old ones, 2 weeks after basolateral amygdala lesions, the changes in ACTH and corticosterone concentrations were more substantial in old rats than in adults. In old rats, ACTH concentration increased by 843 ng/l, and in adult rats by 702 ng/l ($p < 0.01$ between age groups); corticosterone level increased correspondingly by 1200 nmol/l and 842 nmol/l ($p < 0.01$ between age groups). However, the aldosterone concentration was significantly increased in adult rats and did not change in old animals. We have encountered a similar situation in a study of the responses of the adrenal cortex to stress in rats of various ages (Verkhratsky *et al.* 1988). Inasmuch as corticosterone is a precursor for aldosterone, the lack of increase of the concentration of the latter hormone in the blood plasma of old rats following a large surge of corticosterone may indicate depletion of the substrate pool for aldosterone synthesis in the adrenals of old rats.

TSH and thyroxine

Data regarding the influence of limbic structures on the regulation of the pituitary–thyroid axis are scarce. Stimulation of the dorsal hippocampus was reported to increase TSH secretion. The influence of the amygdala on radioactive iodine uptake by the thyroid is stimulatory following low-frequency stimulation, whereas high-frequency stimulation is inhibitory. Experiments involving lesions and stimulation of the medial and basolateral areas of the amygdala indicate that the medial area of the basolateral amygdala facilitates TSH secretion (Chepurnov and Chepurnova, 1981).

In the present experiment (Table 2), 2 weeks after lesioning of the dorsal/ventral hippocampus and amygdala in adult rats, the concentration of TSH in the blood decreased significantly. The most pronounced decrease in TSH levels was observed in animals with a dorsal hippocampus lesion (3.3 mU/l). Less pronounced lowering was

TABLE 2. INFLUENCE OF LIMBIC STRUCTURE LESIONS ON TSH AND THYROXINE CONCENTRATION IN BLOOD PLASMA OF ADULT AND OLD RATS

		TSH (mU/l)		*Thyroxine (nmol/l)*	
Limbic structure	*Age group*	*Initial*	*14 days*	*Initial*	*14 days*
Dorsal hippocampus	Adult	5.9 ± 0.1	2.6 ± 0.04*	106.1 ± 9.0	40.1 ± 2.0*
	Old	5.8 ± 0.1	10.8 ± 0.15*	82.9 ± 4.3	76.1 ± 7.4
			$p < 0.001$	$p < 0.05$	$p < 0.01$
Ventral hippocampus	Adult	15.8 ± 0.1	13.5 ± 0.03*	104.8 ± 8.9	81.1 ± 7.7
	Old	5.9 ± 0.1	8.7 ± 0.15*	62.7 ± 7.4	74.1 ± 9.3†
			$p < 0.001$	$p < 0.01$	
Septum	Adult	16.2 ± 0.1	17.9 ± 0.07*	102.2 ± 9.7	104.7 ± 18.8
	Old	5.9 ± 0.2	11.1 ± 0.18	68.8 ± 6.2	87.3 ± 13.5
			$p < 0.001$	$p < 0.01$	
Amygdala	Adult	15.9 ± 0.04	14.6 ± 0.07*	112.9 ± 12.7	107.5 ± 15.5
	Old	6.1 ± 0.1	44.8 ± 0.31*	83.4 ± 12.0	161.9 ± 8.1†
			$p < 0.001$		$p < 0.02$

*$p < 0.001$; †$p < 0.01$ = significant changes in the same age group.
p = significant difference between age groups.

observed in rats with ventral hippocampus lesions (2.3 mU/l) and amygdala lesions (1.2 mU/l). At the same time, in septum-lesioned adult rats, the level of TSH in plasma increased (1.7 mU/l). In accordance with the shifts of TSH levels, the concentration of thyroxine in blood plasma also changed (Table 2).

In contrast to the results obtained in adult animals, in old rats, 2 weeks after lesion of limbic system structures, the concentration of TSH in the blood increased significantly: in ventral hippocampus, lesioned rats by 2.7 mU/l, and in dorsal hippocampus and septum, by 5.0 mU/l and 5.2 mU/l, respectively. But the most pronounced increase in TSH concentration occurred in old rats 2 weeks after basolateral amygdala electrocoagulation (38.7 mU/l). However, the thyroxine concentration in the plasma of old rats did not change in response to changes in TSH, as was observed in adult animals. In old lesioned animals with a significant increase in TSH secretion (dorsal and ventral hippocampus and amygdala), the responses of thyroxine secretion were variable. Following dorsal hippocampus lesioning, the thyroxine concentrations fell (6.8 nmol/l) but rose following ventral hippocampus lesioning (11.4 nmol/l). Furthermore, in old animals, following lesioning of the amygdala, the concentration of thyroxine rose dramatically (78.5 nmol/l).

CONCLUSIONS

The data obtained have shown that, with age, significant changes occur regarding the limbic system control of the hypothalamic–pituitary–adrenal/thyroid axes in male rats. Following bilateral lesions in the dorsal/ventral hippocampus and septum, plasma concentrations of ACTH, TSH, corticosterone, aldosterone, and thyroxine were altered. Comparison of the changes in plasma levels of the examined hormones in old vs. adult rats indicates that the influence of the dorsal/ventral hippocampus and septum on

hypothalamic/pituitary function tends to diminish as age increases. It appears that the age-related decline in the regulatory role of limbic structures is most pronounced for the ventral hippocampus, with changes in the influence of the dorsal hippocampus and septum following in a descending order. However, just the opposite is observed with the amygdala, in that the influence of this region of the limbic system on the hypothalamus and pituitary appears to become enhanced during the aging process.

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