Resistance to Neurodegenerative Brain Damage in August and Wistar Rats

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In Wistar and August rats characterized by different resistance to acute emotional stress we compared the resistance to neurodegenerative brain damage (model of Alzheimer's disease) produced by administration of a neurotoxic peptide fragment (25-35) β-amyloid into the brain. August rats were more resistant to acute stress and development of neurodegenerative disorders compared to Wistar rats. This conclusion was derived from studying animal behavior in conditioned passive avoidance task and open-field test that characterize cognitive function of the brain. Administration of β-amyloid modulated the behavior of Wistar rats, which reflected the impairment of memory and orientation and exploratory activity in these animals. These disturbances in Wistar rats were accompanied by activation of lipid peroxidation in the hippocampus.

Key Words: *August and Wistar rats; β-amyloid; Alzheimer's disease; cognitive functions; neurodegenerative damage*

August rats are more resistant to emotional stress than Wistar rats [5]. Stress exposure induces a weaker stress response in August rats, which is accompanied by less severe damage (compared to Wistar rats) [6]. In August rats, activity of the stress-limiting system that decreases the stress response and attenuates stress-induced changes is higher than in Wistar rats [7,4]. Since emotional stress plays an important role in the pathogenesis of neurodegenerative disorders, including Alzheimer's disease (AD) [12,13,14], we hypothesized that genetically determined increase in organism's resistance to stress can counteract the development of this disorder. Published data show that the incidence of acute myocardial infarction in August rats is lower than in Wistar rats [2]. It should be emphasized that the development of this disease is closely related to emotional stress. In light of this we compared the resistance of Wistar and August rats to experimental neurodegenerative damage (AD) [10]. Oxidative stress plays a key role in the pathogenesis of stress-induced and neurodegenerative changes [9,11]. In the present study the intensity of free radical reactions in brain structures was compared in Wistar and August rats with experimental AD.

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MATERIALS AND METHODS

Experiments were performed on age-matched male Wistar (320±50 g) and August rats (250±30 g). AD was modeled by bilateral administration of a neurotoxic β-amyloid peptide fragment (25-35) into *n. basalis magnocellularis*. The preparation (2 μl, 0.4×10^{-9} M) was stereotactically administered into each cerebral hemisphere [10]. Some August and Wistar rats were sham-operated, *i.e.* were treated similarly to animals with experimental AD, but received physiological saline instead of β-amyloid. The resistance of animals to neurodegenerative damage was evaluated by the incidence of memory disorders (marker of clinical and experimental AD) and changes in the open-field

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behavior (cognitive function). The severity of memory impairment was evaluated by studying the conditioned passive avoidance response (CPAR) [14]. According to the results of previous studies, this test was performed 14 days after β-amyloid administration [10].

CPAR was studied in a special setup. The rat was placed in a restricted illuminated area. The latency of transition to the dark chamber was recorded over 3 min (LP_1). The animal entering the dark chamber received electroshock and was removed from the setup. The rat was tested after 24 h under similar conditions. The latency of transition to the dark chamber was recorded over 3 min (LP_2). This period reflects storage in memory or learning. The better was the storage of information about electroshock, the longer were LP_2 and LP_2-LP_1 difference (ΔLP, retention score).

Open-field behavior was studied 14 days after CPAR task. Horizontal (locomotion on the floor) and vertical activity (rearing postures without leaning on the wall) was recorded over 2 min. The degree of oxidative stress in the frontal and parietal cortex and hippocampus (memory-related structures) was estimated by the concentration of lipid peroxidation (LPO) products. We measured the concentration of thiobarbituric acid (TBA)-reactive substances. The rats were decapitated 2 days after the last behavioral trial. Brain structures were isolated under cold conditions and stored in liquid nitrogen until the analysis of TBA-reactive products. The amount of TBA-reactive products was routinely measured in tissue homogenates taking into account that the major product of TBA-reaction is MDA—TBA complex. Optical density of products was recorded on a KFK-3 photometer at 515, 532, and 550 nm. The concentration of these products was expressed in nmol MDA per 1 g tissue. We performed 6 experimental series (3 series with August rats and 3 series with Wistar rats): intact animals (control), β-amyloid treatment, and sham operation.

The results were analyzed by pairwise Wilcoxon *T* test (comparative analysis of LP_1 and LP_2, calculation of ΔLP in a CPAR task for each animal) and nonparametric Wilcoxon—Mann—Whitney *U* test (comparative analysis of the data on CPAR task and open-field test in different series for different strains). Statistical treatment of data on the LPO intensity was performed using Student's *t* test.

RESULTS

CPAR testing showed that 90% control August rats and 90% control Wistar rats "remember" electroshock. The retention score (ΔLP) in Wistar rats was higher than in August rats. Administration of β-amyloid impaired memory process in Wistar rats: the difference between LP_1 and LP_2 was statistically insignificant ($p>0.05$, pairwise Wilcoxon *T* test). Therefore, the majority of rats did not "remember" electroshock. The ratio of "remembering" animals was 37% (*vs*. 90% in the control). As differentiated from Wistar rats, β-amyloid treatment did not disturb memory processes in August rats. This conclusion was derived from studying of the number of "remembering" animals and retention score (ΔLP). The study of indexes for memory impairment suggests that in August rats higher resistance to acute stress [4,5] is associated with higher resistance to neurodegenerative damage.

In the control series, horizontal locomotor activity in the open field did not differ in August and Wistar rats. The index of vertical activity in August rats was higher than in Wistar rats (Table 1). These data indicate that August rats are characterized by a higher orientation and exploratory activity, which agrees with published data [5]. β-Amyloid treatment increased horizontal (locomotion) and vertical activity in August rats. However, in Wistar rats β-amyloid decreased horizontal activity by 41% (it became 3-fold lower

TABLE 1. Effect of β-Amyloid on CPAR Performance and Open-Field Behavior of Wistar and August Rats

Group		CPAR task (mean values)		Open-field test (mean values)	
		ΔLP, sec	electroshock-remembering rats, %	horizontal activity (number of crossed squares)	vertical activity (total number of rearing postures)
Control	August	100.6^{xx}	90	25.2	11.4
	Wistar	142.7^{*xx}	90	25.6	8.6*
β-Amyloid	August	89.9^{x}	89	36.2^{+}	15.2^{+}
	Wistar	36.1^{++*}	37^{++*}	26.8*	5.1^{++*}
Sham operation	August	96.2	100	26.4	11.6
	Wistar	103	89	44.2^{+*}	8.1*

Note. $^{*}p<0.01$ compared to August rats; $^{+}p<0.05$ and $^{++}p<0.01$ compared to the control (Wilcoxon—Mann—Whitney *U* test); $^{x}p<0.05$ and $^{xx}p<0.01$, significant differences between ΔLP (pairwise Wilcoxon *T* test).

TABLE 2. Effect of β-Amyloid on MDA Concentration in Brain Structures of Wistar and August Rats (nmol/g tissue, *M*±*m*)

Group	Brain structures	
	hippocampus	cortex
Control		
August	70.15±3.40 *n*=16	64.40±3.90 *n*=16
Wistar	71.20±1.84 *n*=10	79.26±4.2* *n*=8
β-Amyloid		
August	72.6±4.4 *n*=16	64.50±3.6 *n*=16
Wistar	77.8±2.51^{+} *n*=10	77.0±3.26* *n*=9
Sham operation		
August	73.65±13.50 *n*=8	65.68±4.52 *n*=8
Wistar	71.25±2.00 *n*=8	84.41±8.40* *n*=9

Note. *n*, number of rats. $p<0.05$: *compared to August rats; $^{+}$compared to the control (Student's *t* test).

than in August rats), and did not change horizontal activity. These findings indicate that orientation and exploratory activity reflecting cognitive function of the brain decreased in Wistar rats, but increased in August rats with experimental AD. It should be emphasized that sham-operated rats did not differ from control animals by behavioral characteristics. Hence, the observed changes are caused by the test peptide, but not surgery or injection into the brain.

Comparison of the intensity of free radical oxidation in brain structures revealed no differences in MDA concentration in the cerebral cortex of treated and control August and Wistar rats. Therefore, β-amyloid had no effect on LPO in this brain structure. However, the intensity of LPO in the cerebral cortex of Wistar rats was higher than in August rats. In the hippocampus, administration of β-amyloid increased MDA concentration only in Wistar rats (by 9% compared to the control). However, we found no differences in hippocampal MDA concentration in Wistar and August rats (Table 2). These data show that lower resistance to neurodegeneration processes in Wistar rats (judging from behavioral changes) is accompanied by activation of free radical oxidation in the hippocampus.

Thus we found that August and Wistar rats characterized by genetically determined differences in the resistance to emotional stress demonstrate also different resistance to experimental neurodegenerative damage (judging from CPAR task and open-field test performance). Wistar rats are less resistant to both acute stress and neurodegenerative processes: they demonstrate serious memory disorders in CPAR task and decrease in orientation and exploratory activity in the open field. These changes were not observed in stress-resistant August rats. Since stress resistance is determined by activity of the stress-limiting system decreasing stress response and attenuating stress-induced changes, our results suggest that resistance to experimental neurodegenerative process and AD depends on activity of this stress-limiting system. Spontaneous vertical activity in the open-field test (orientation and exploratory activity) is higher in August rats and depends on the intensity of dopamine-involving processes [3]. Published data show that activity of the stress-limiting dopaminergic system in August rats is higher than in Wistar rats. [7].

Nitric oxide (NO) plays an important role in the pathogenesis of neurodegeneration. Our previous studied showed that August rats are characterized by higher activity [7] of the stress-limiting antioxidant system and higher resistance to oxidative stress during cardiac ischemia and reperfusion [1]. This conclusion is supported by the data on the intensity of lipid peroxidation in brain structures of Wistar and August rats receiving β-amyloid (Table 2). In our experiments LPO intensity underwent less significant changes. It should be emphasized that LPO was studied 1 month after β-amyloid administration. In this period oxidative stress that plays an important role in the pathogenesis of neurodegeneration was partially compensated. Further studies are required to evaluate the intensity of LPO over the first week after the incidence of AD.

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