Antiamnestic Effect of α7-Nicotinic Receptor Agonist RJR-2403 in Middle-Aged Ovariectomized Rats with Alzheimer Type Dementia

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The effects of chronic combined treatment with α7-nicotinic cholinergic receptor agonist RJR-2403 (1.0 mg/kg intraperitoneally) or α7-nicotinic cholinergic receptor antagonist mecamylamine (1.0 mg/kg intraperitoneally) and 17β-estradiol (0.5 μg per rat intramuscularly) for 10 days on passive avoidance retention were studied in middle-aged (15 months) ovariectomized rats with experimental Alzheimer type dementia. Chronic treatment with RJR-2403 and 17β-estradiol had a pronounced antiamnestic effect under conditions of Alzheimer type dementia in middle-aged ovariectomized rats.

Key Words: *α7-nicotinic choplinoreceptors (RJR-2403); mecamylamine; oophorectomy; memory; Alzheimer's disease*

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by disorders in cognitive functions and memory [10]. The disease is characterized by pronounced hypofunction of the cholinergic system and reduction in activities of other neuromediator systems [4]. Stimulation of central α7- and α4β2-subtypes of nicotinic cholinergic receptors protects the neurons from the toxic effects of β-amyloid and excitotoxicity of stimulatory amino acids [4,8,10].

Numerous clinical and experimental data indicate a functional relationship between the cholinergic and pituitary-ovarian systems in the realization of the cognitive functions of the brain [11,12]. It was hypothesized that disorders in the cholinergic system associated with estrogen deficiency promote the development of AD [6,7].

We carried out a comparative analysis of activation and blockade of α7-nicotinic cholinergic receptors during conditioned reflectory activity in middle-aged ovariectomized rats with experimental Alzheimer type dementia (ATD).

MATERIALS AND METHODS

The study was carried out on 15-month-old female Wistar rats (n=120; 280-320 g) from Rappolovo Breeding Center. The animals were kept in a vivarium under natural illumination at standard temperature and had free access to water and standard fodder. The studies were carried out at 9.00-12.00.

ATD was induced as follows. β-Amyloid was dissolved in water (3 μg/ml). The space above the 4th ventricle was cleaned, the bone was drilled, and a cannula was inserted into the ventricle. The coordinates were determined according to the Atlas [5]. β-Amyloid (15 μg, 5 μl) was administered through the cannula to experimental animals, controls received 5 μl water for injections. Behavioral experiments were started on day 21 after intracerebroventricular injection.

For behavioral test the rats were divided into the following groups (10-12 per group): 1) intact females injected with saline (control); 2) intact fe-

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males injected with β-amyloid; 3) intact females with ATD injected with RJR-2403 (1.0 mg/kg intraperitoneally for 10 days); 4) intact rats with ATD injected with mecamylamine antagonist (1.0 mg/kg intraperitoneally for 10 days); 5) ovariectomized (OE) rats; 6) OE rats treated with β-amyloid; 7) OE rats with ATD treated with 17β-estradiol (0.5 μg/rat intramuscularly daily for 10 days); 8) OE rats with ATD treated with RJR-2403 for 10 days; 9) OE females with ATD treated with RJR-2403 in combination with estradiol; 10) OE females with ATD treated with mecamylamine for 10 days; and 11) OE rats with ATD treated with mecamylamine in combination with estradiol.

The ovaries were removed by the standard method [2]. The efficiency of exogenous estradiol in OE females was evaluated by vaginal smears.

Retention of memory trace under conditions of experimental ATD was evaluated in the conditioned passive avoidance test [1]. Passive avoidance was conditioned using a single electrocutaneous support in a device consisting of two chambers: large (illuminated) and small (dark) with electrified floor connected by a round hole. During training the rat was placed for 3 min into the center of illuminated chamber with its tail to the hole into the dark chamber. The animal examined the chamber, found the hole, and run into the dark chamber. Due to its biological peculiarities (congenital preference of dark sites in rodents), the rat preferred the dark chamber and spent there about $^2/_3$ of time. The latency of entry into the dark chamber was registered. At the end of the 3rd minute, electric current (50 Hz, 6 mA) was passed through the electric floor of the dark chamber, which forced the rat to run into the illuminated chamber, from which it was removed. In this case the reaction was considered to be conditioned. Passive avoidance performance was evaluated after 24 h. The rat was put into the device for 3 min. The same parameter was recorded as during training. If the rat did not enter the dark chamber, it was regarded as the reproduction of passive avoidance habit, while shortening the time spent in the illuminated room was considered as the loss of habit.

The data were statistically processed by means of ANOVA test at $p<0.05$ using Statistica software.

RESULTS

Passive avoidance testing 24 h after preliminary training showed that β-amyloid injected to middle-aged females with ATD (group 2) several-fold shortened the duration of rat stay in the light chamber (13.5±1.8 sec, $p<0.05$) compared to intact females

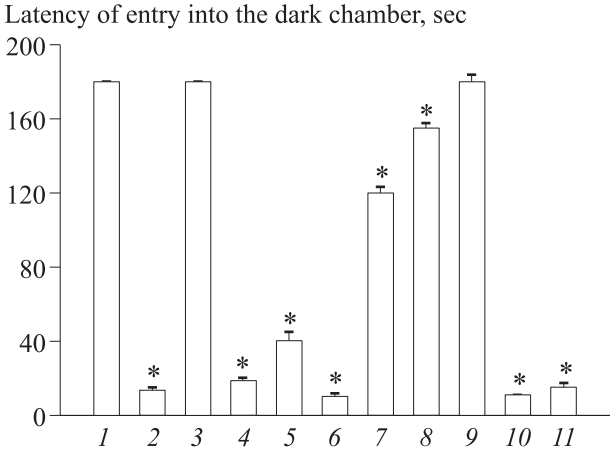

Fig. 1. Effects of chronic treatment with RJR-2403, mecamylamine, or their combinations with 17β-estradiol on retention of PACR in middle-aged OE female rats with experimental ATD. *1*) intact females (control); *2*) intact females treated with β-amyloid; *3*) intact females injected with β-amyloid and RJR-2403; *4*) intact females injected with β-amyloid and mecamylamine; *5*) OE females; *6*) OE females injected with β-amyloid; *7*) OE females injected with β-amyloid and 17β-estradiol; *8*) OE females treated with β-amyloid and RJR-2403; *9*) OE females injected with β-amyloid, RJR-2403, and 17β-estradiol; *10*) OE females injected with β-amyloid and mecamylamine; *11*) OE females treated with β-amyloid, mecamylamine, and 17β-estradiol. *$p<0.05$ compared to the control.

injected with saline (control), which stayed in illuminated chamber during the entire test period (180 sec) (Fig. 1). Group 3 animals receiving RJR-2403 demonstrated passive avoidance behavior ($p<0.05$), while group 4 rats treated with mecamylamine exhibited no reaction of this kind.

Group 6 rats injected with β-amyloid also demonstrated complete amnesia of the habit (10.2±1.4 sec in the light chamber, $p<0.05$ compared to control group 1). Ovariectomized rats with ATD injected with 17β-estradiol (group 7) partially retained passive avoidance habit (120.0±3.4 sec in the light chamber; $p<0.05$) in comparison with group 1 (control). Passive avoidance habit was partially retained in OE females (group 8; 155.0±2.6 sec in the light chamber, $p<0.05$), while after combined treatment with RJR-2403 and 17β-estradiol (group 9) the animals completely reproduced the conditioned reaction (180.0±3.8 sec). Mecamylamine injected alone or in combination with 17β-estradiol (groups 10 and 11) had no positive effect on passive avoidance performance.

Chronic treatment with α7-nicotinic cholinoreceptor agonist or antagonist had opposite effects on passive avoidance retention in middle-aged OE females with experimental ATD. Combined treatment with RJR-2403 and 17β-estradiol produced an antiamnestic effect (normalized the reproduction of the memory trace under conditions of experimental ATD in middle-aged OE females). Potentiation of the positive effects of RJR-2403 and 17β-

estradiol on the retention of memory trace was observed in OE females injected with β-amyloid. Mecamylamine injected alone or in combination with 17β-estradiol did not improve passive avoidance performance in OE females with ATD.

According to published data, activation of central α7-nicotinic cholinergic receptors protects the neurons from toxic effect of β-amyloid [4,10]. *In vitro* experiments showed that estrogens reduce neurotoxicity of β-amyloid by reacting with nicotinic cholinergic receptors [4,10]. In addition, the positive effect of estrogens on the cognitive functions under conditions of AD can be due to their antioxidant properties and to activation of expression of NF-kB factor, associated with induction of antioxidant enzymes [9]. Our findings also indicate that injection of 17β-estradiol alone as replacement therapy to middle-aged rats is insufficient for complete recovery of memory trace retrieval in ATD. The sensitivity of estrogen receptors to estradiol in females decreases with age; long-lasting estrogen deficiency in old females induced by ovariectomy in the absence of timely and long-term replacement therapy decreases estradiol-binding capacity of estrogen receptors [3]. These data suggest that purposeful combined pharmacological modulation of α7-nicotinic cholinergic receptors and estrogen receptors leads to restoration of cognitive processes in middle-aged OE females with ATD.

Hence, chronic treatment with RJR-2403 and 17β-estradiol had a pronounced antiamnestic effect in middle-aged OE rats with ATD.

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