PHARMACOLOGY AND TOXICOLOGY

Antiamnesic Effect of Combined Treatment with Galantamine and Estradiol in Middle-Aged Ovariectomized Female Rats

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The effect of chronic combined treatment with galantamine and 17β-estradiol on passive avoidance retention was studied in middle-aged ovariectomized female rats (15 months) with scopolamine-induced amnesia. Combined treatment with galantamine and estradiol completely restored retrieval of memory traces in middle-aged ovariectomized female rats.

Key Words: *ovariectomy; galantamine; pharmacological amnesia; age*

The cholinergic system determines the type of memory processes. Previous studies identified optimal activity of central cholinoceptors for adequate perception and evaluation of environmental information [10]. Any deviation from the optimal activity of central cholinoceptors impairs acquisition and performance. The existence of functional relationships between the cholinergic and hypothalamic-pituitary-ovarian system in cognitive function of the brain was shown in clinical and experimental studies [3,6]. Abnormalities of the cholinergic system during estrogen deficiency can contribute to the development of Alzheimer's disease [3,7].

Here we studied the effect of a cholinesterase inhibitor galantamine administered alone or in combination with 17β-estradiol on passive avoidance conditioning in middle-aged ovariectomized female rats (15 months) with pharmacological amnesia.

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MATERIALS AND METHODS

Experiments were performed on 120 adult female outbred albino rats weighing 280-320 g (Rappolovo nursery). The animals were maintained in a vivarium under natural light/dark cycle and standardized temperature and feeding conditions. They had free access to water and food. The study was conducted at 9.00-12.00. The rats were divided into groups (10-20 animals per group). Group 1 comprised intact females receiving physiological saline (control I). Group 2 included intact rats receiving subcutaneous injection of scopolamine in a dose of 1 mg/kg 30 min before the start of learning (pharmacological model of amnesia). Group 3 consisted of ovariectomized females that were examined 28 days after surgery (control II). Group 4 ovariectomized females (21 days postoperation) received intraperitoneal injections of 17β-estradiol in a daily dose of 5 μg in 0.5 ml oil for 7 days. Group 5 ovariectomized females (21 days postoperation) received intraperitoneal injections of galantamine hydrobromide in a daily dose of 1 mg/kg for 7 days. Group 6 ovariectomized females (21 days

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postoperation) received intraperitoneal injections of galantamine hydrobromide and 17β-estradiol in daily doses of 1 mg/kg and 5 μg in 0.5 ml oil, respectively, for 7 days. Scopolamine hydrobromide in a dose of 1 mg/kg was injected subcutaneously to 50% animals of groups 3, 4, 5, and 6 (10 of 20 rats) for modeling experimental amnesia. The rats were treated 30 min before the start of learning. The rest animals of these groups did not receive scopolamine hydrobromide and were tested for memory retention on the model of conditioned passive avoidance response (CPAR).

Ovariectomy was performed routinely [1]. The effectiveness of exogenous 17β-estradiol in ovariectomized females was estimated by examination of vaginal smears.

Retention of memory trace in animals with scopolamine-induced amnesia was estimated by CPAR [2].

The results were analyzed by nonparametric analysis of variance using Statistica 4.0 software.

RESULTS

Study on the model of CPAR showed that intact rats exhibit passive avoidance retention 24 h after learning. These animals remained in the light compartment over 180 sec (Fig. 1). Ovariectomy significantly decreased the time spent in the light compartment during repeated testing (94.4±4.9 sec, $p<0.05$ compared to control group I). Chronic treatment with galantamine did not improve CPAR performance in middle-aged ovariectomized females (106.3±8.6 sec) compared to control ovariectomized rats. Administration of 17β-estradiol alone or in combination with galantamine improved CPAR retention in ovariectomized females. Similarly to intact rats of the control group, these females remained in the light compartment over 180 sec.

Further experiments were performed on the model of scopolamine-induced amnesia 24 h after learning. The time spent by intact rats in the light compartment decreased by several times after scopolamine administration (13.5±1.68 *vs*. 180 sec in animals of control group I, $p<0.05$; Fig. 2). Administration of scopolamine to middle-aged ovariectomized females 30 min before learning decreased the time spent in the light compartment to 10.2±0.4 sec ($p<0.05$ compared to control group II). CPAR performance was observed in 30% ovariectomized females aging 15 months and receiving the standard replacement therapy with 17β-estradiol. Administration of galantamine slightly improved CPAR performance in ovariectomized rats with scopolamine-induced amnesia. The time spent by animals of this group in the light compartment was 98.0±6.4 sec. Combined treatment with galantamine and 17β-estradiol was followed by complete recovery of CPAR performance in rats with scopolamine-induced amnesia. The time spent by these specimens in the light compartment was 180 sec.

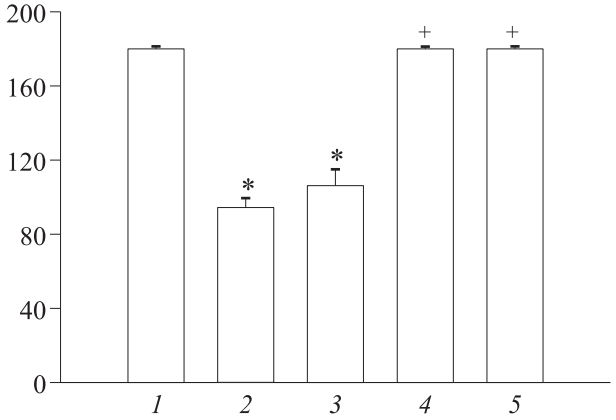

Fig. 1. Effect of chronic treatment with galantamine alone or in combination with estradiol on passive avoidance retention in middle-aged ovariectomized female rats: intact females (*1*); ovariectomy (*2*); ovariectomy+galantamine (*3*); ovariectomy+17β-estradiol (*4*); ovariectomy+17β-estradiol+galantamine (*5*). Here and in Fig. 2: ordinate, latency of the entrance into the dark compartment (sec). $p<0.05$: *compared to intact rats; +compared to ovariectomy.

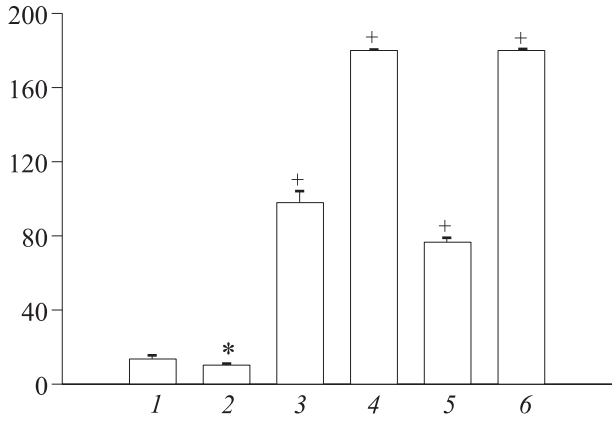

Fig. 2. Effect of chronic treatment with galantamine alone or in combination with estradiol on passive avoidance retention in middle-aged ovariectomized female rats with scopolamine-induced amnesia: intact females+scopolamine (*1*); ovariectomy+scopolamine (*2*); ovariectomy+galantamine+scopolamine (*3*); ovariectomy+17β-estradiol+scopolamine (30%, *4*; 70%, *5*); ovariectomy+17β-estradiol+galantamine+scopolamine (*6*). $p<0.05$: +compared to ovariectomy+scopolamine.

The antiamnesic effect was observed after combined treatment with galantamine and estradiol. These drugs normalize memory performance in middle-aged ovariectomized rats with pharmacological amnesia. Estrogen deficiency is accompanied by dysfunction of cholinergic neurons in rat brain. Published data show that ovariectomy is followed by inhibition of acetylcholinesterase, decrease in expression of acetylcholinesterase mRNA in the

hippocampus, frontal cortex, and neocortex [7,8], impairment of neuronal transport of choline precursors, and reduction of K^+-dependent acetylcholine release [5]. The concentration and activity of acetylcholinesterase in young ovariectomized female rats increase over the first 10 days of estradiol treatment. These changes are accompanied by regeneration of cholinergic neurons. After 2-week replacement therapy with estradiol, activity of the cholinergic system in animals does not differ from that in young intact females [4,5].

Our results indicate that replacement monotherapy with estradiol is insufficient for complete recovery of memory trace retrieval in middle-aged ovariectomized rats. These females are characterized by age-related decrease in the sensitivity of estrogen receptors for estradiol. Long-term estrogen deficiency in old rats associated with ovariectomy and not corrected by replacement therapy decreases estradiol-binding activity of estrogen receptors [2,4,5]. These data suggest that replacement monotherapy with estradiol for normalization of memory processes in middle-aged ovariectomized female rats does not recover adequate perception and evaluation of environmental information via central cholinoceptors.

We conclude that chronic combined treatment with galantamine and 17β-estradiol has a strong antiamnesic effect in middle-aged ovariectomized female rats with scopolamine-induced amnesia.

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