Combined Administration of NAN-190 and 17β-Estradiol Abolishes the Amnesic Effect of Scopolamine in Middle-Aged Ovariectomized Rats

N. S. Sapronov, Yu. O. Fedotova, and N. A. Losev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 7, pp. 90-93, July, 2006
Original article submitted August 30, 2005

Chronic combined administration of 5-HT_{1A} receptor antagonist NAN-190 and 17β-estradiol improved conditioned passive avoidance performance in middle-aged ovariectomized rats with scopolamine-induced amnesia. Our results suggest that the ovarian hormonal, cholinergic, and serotoninergic systems play a role in age-related cognitive disturbances under conditions of hypoestrogenic syndrome.

Key Words: *5-HT_{1A} receptors; 8-OH-DPAT; NAN-190; ovariectomy; scopolamine*

The central cholinergic system plays a fundamental role in cognitive function of the brain [2,12]. Changes in activity of central cholinoceptors from the optimal value impair skill acquisition and performance, which is typical of age-related disturbances and Alzheimer's disease [12].

Recent studies showed that cognitive deficit cannot be related exclusively to dysfunction of the cholinergic system [2]. Aging and Alzheimer's disease are accompanied by hypofunction of various neurotransmitter systems, including the glutamatergic, GABAergic, and monoaminergic system (*e.g.*, serotoninergic system). These changes have a negative effect on higher nervous activity [8]. Subtype 1A serotonin receptors (5-HT_{1A} receptors) and cholinoceptors are involved in the mechanisms of spatial memory and avoidance behavior [6]. These receptors modulate the release of acetylcholine in brain structures [7,9].

Clinical and experimental studies demonstrated the existence of functional relationships between the cholinergic/serotoninergic and hypothalamic-pituitary-ovarian system in cognitive function of the brain [3,4,10]. Dysfunction of the cholinergic and serotoninergic systems under conditions of estrogen deficiency contributes to the development and progression of Alzheimer's disease [5,10].

Taking into account the existence of close relationships between hormonal, cholinergic, and serotoninergic neurotransmitter systems, it is important to study the role of 5-HT_{1A} receptors in avoidance behavior under conditions of estrogen deficiency.

This work was performed to study conditioned activity of middle-aged ovariectomized (OE) rats with scopolamine-induced amnesia. 5-HT_{1A} receptor agonist 8-OH-DPAT and 5-HT_{1A} receptor antagonist NAN-190 were administered alone or in combination with 17β-estradiol for 14 days.

MATERIALS AND METHODS

Experiments were performed on 100 female middle-aged Wistar rats (15 months, 280-320 g) obtained from the Rappolovo nursery. The animals were kept in a vivarium under the natural light/dark regimen, standard temperature and feeding conditions, and *ad libitum* water and food supply. The study was conducted at 9.00-12.00. Before behavioral tests the rats were divided into groups (8-10 animals per group): intact females, physiological saline (group 1); OE females, oil solvent

S. V. Anichkov Department of Neuropharmacology, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg

intramuscularly (group 2); intact females, scopolamine hydrobromide (SG, 1 mg/kg, Sigma) subcutaneously 30 min before behavioral testing (group 3); OE females, SG injection 30 min before behavioral testing (group 4); intact females, 8-OH-DPAT (0.05 mg/kg, Sigma) subcutaneously, 14 days (group 5); intact females, NAN-190 (0.1 mg/kg, Sigma) intraperitoneally, 14 days (group 6); OE females, 17β-estradiol (0.5 mg, Sigma) intramuscularly, 14 days (group 7); OE females, daily injections of 8-OH-DPAT, 14 days (group 8); OE females, daily injections of 8-OH-DPAT and 17b-estradiol (0.5 mg), 14 days (group 9); OE females, daily injections of NAN-190, 14 days (group 10); OE females, daily injections of NAN-190 and 17β-estradiol (0.5 mg), 14 days (group 11). Amnesia in animals of groups 5-11 was induced by administration of SG 30 min before learning.

Ovariectomy was performed routinely. The test preparations were started 2 weeks after surgery. Treated and control females were examined during the diestrus phase. This physiological state is characterized by a balanced hormonal status. The effects of exogenous 17β-estradiol in OE females were evaluated by examination of vaginal smears.

Retention of memory traces in animals with scopolamine-induced amnesia was estimated by the conditioning passive avoidance response (CPAR) with single reinforcement [1].

The results were analyzed by one-way analysis of variance (ANOVA, post-hoc, Tukey test). The differences were significant at $p<0.05$.

RESULTS

The study on the model of scopolamine-induced amnesia showed that SG administration to intact females 24 h after learning several times decreases the time spent in the light compartment. It should be emphasized that group 1 females remained in this compartment over 180 sec (Fig. 1, *a*). Ovariectomy had a significant negative effect on CPAR performance in rats compared to group 1 animals. SG administration completely suppressed CPAR performance in rats compared to group 2 animals (Fig. 1, *b*).

Chronic administration of 8-OH-DPAT to intact females of group 5 potentiated the amnesic effect of SG. It manifested in a significant decrease in the time spent by rats in the light compartment (compared to group 3 animals). However, NAN-190 increased the time spent by intact SG-receiving females of group 6 in the light compartment (compared to group 3 animals). However, group 6 rats spent a lower period in the light compartment compared to group 1 animals.

CPAR performance was observed only in 30% 15-month-old OE rats with scopolamine-induced amnesia (group 7) receiving replacement therapy with 17β-estradiol.

Administration of 8-OH-DPAT alone or in combination with 17β-estradiol had no effect on CPAR performance in middle-aged OE rats with scopolamine-induced amnesia (compared to group 4 animals, Fig. 1, *b*). By contrast, chronic administration of NAN-190 significantly improved memory performance in OE rats with scopolamine-induced amnesia (group 10) compared to group 4 animals. However, the time spent in the light compartment did not differ in OE females receiving NAN-190 and 17β-estradiol (group 11) and group 1 rats.

These data suggest that stimulation and blockade of 5-HT_{1A} receptors upon treatment with mus-

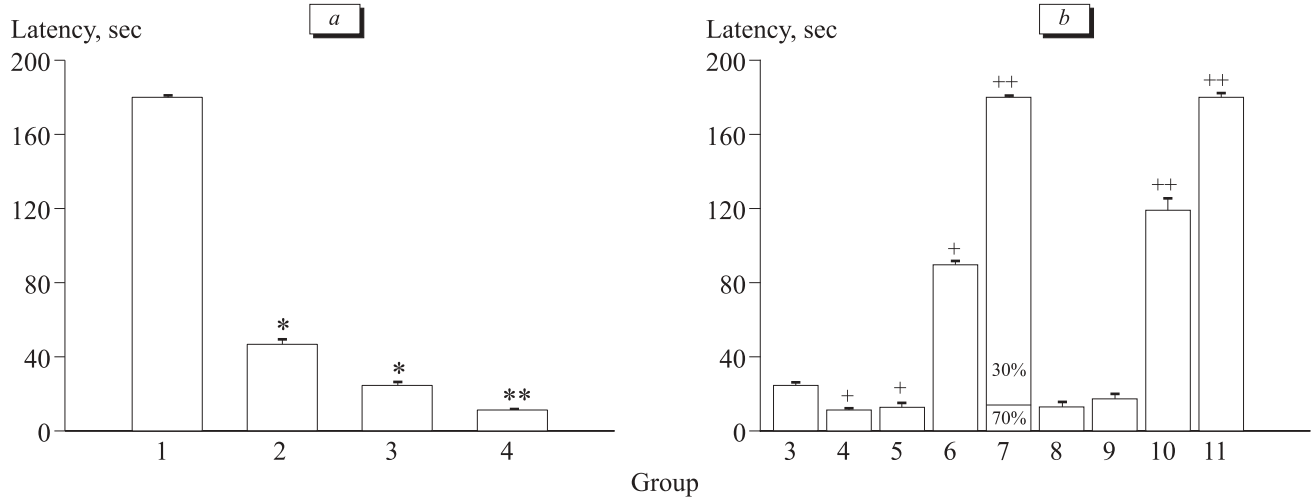

Fig. 1. Effects of SG (*a*) and NAN-190 (*b*) on CPAR retention in middle-aged intact and OE rats. $p<0.05$: *compared to group 1; **compared to group 2; +compared to group 3; ++compared to group 4.

carinic cholinoceptor antagonists (scopolamine-induced amnesia) have opposite effects on passive learning in intact and OE rats. 5-HT_{1A} receptor agonist potentiated the amnesic effect of SG. By contrast, administration of 5-HT_{1A} receptor antagonist produced an antiamnesic effect in CPAR paradigm after treatment with SG. Our results are consistent with published data that combined administration of 5-HT_{1A} receptor agonists and SG has a strong negative effect on conditioned behavior in male rats after spatial and non-spatial learning [6,9]. Moreover, antagonists of 5-HT_{1A} or 5-HT_{2A} receptors improve CPAR in middle-aged and old male rats [6,7].

8-OH-DPAT did not potentiate the amnesic effect of SG under conditions of estrogen deficiency. It was probably related to changes in hormonal status and low concentration of progesterone in the blood of OE rats. Previous studies showed that progesterone impaired cognitive function [3]. Individual or combined administration of NAN-190 and 17β-estradiol has a strong antiamnesic effect on CPAR during scopolamine-induced amnesia. Memory performance in adult OE rats with pharmacological amnesia returned to normal after this treatment. Functional relationships exist between the cholinergic/serotoninergic and hypothalamic-hypophyseal-ovarian system in cognitive functions of the brain [3,10]. Dysfunction of the cholinergic and serotoninergic system under conditions of estrogen deficiency contributes to the development and progression of Alzheimer's disease [5,10].

Our results suggest that replacement monotherapy with estradiol is insufficient for compensation of memory impairment in middle-aged OE rats. Previous studies showed that the sensitivity of estrogen receptors to estradiol in females decreases during aging. Long-term estrogen deficiency in old females subjected to ovariectomy and not receiving replacement therapy contributes to the decrease in estradiol-binding activity of estrogen receptors [3]. We conclude that replacement monotherapy with estradiol for improving memory in adult OE females cannot optimize function of central cholinoceptors for adequate perception and evaluation of environmental information.

The cholinergic and serotoninergic systems reciprocally regulate various behavioral processes [6,8,12]. Anatomically, these systems interact at the level of cholinergic neuronal bodies and dendrites in the forebrain. They modulate functional activity of thalamic, cortical, and hippocampal neurons through various subtypes of serotonin receptors [9]. Biochemical studies showed that the compounds binding to serotonin receptors can activate or inhibit acetylcholine influx/release in cortical neurons [7,9]. It can be hypothesized that a 5-HT_{1A} receptor antagonist NAN-190 facilitates acquisition and retention of memory traces by modulating activity of muscarinic cholinoceptors or acetylcholine release from hippocampal neurons, because hippocampus plays an important role in the regulation of learning and memory [6]. 5-HT_{1A} receptor blockade with SG probably causes an imbalance between functions of the serotoninergic and cholinergic system. 5-HT_{1A} receptors mediate the influence of estradiol on the serotoninergic system [3]. Combined treatment with NAN-190 and estradiol under conditions of estrogen deficiency and muscarinic cholinoceptor blockade can prevent dysfunction of the hormonal and neurotransmitter systems. Hence, chronic combined administration of NAN-190 and 17β-estradiol has a strong antiamnesic effect in middle-aged OE rats with scopolamine-induced amnesia.

Our results provide the basis for the development of new approached to the use of 5-HT_{1A} receptor antagonists and estradiol for pharmacotherapy of cognitive disorders during aging or in Alzheimer's disease associated with ovarian hypofunction.

This work was supported by the Russian Foundation for Basic Research (grant No. 04-04-49025).

REFERENCES

1. Ya. Buresh, O. Bureshova, and J. P. Houston, *Methods and Main Experiments in Studying the Brain and Behavior* [in Russian], Moscow (1991).
2. Y. Abe, A. Aoyagi, T. Hara, *et al*., *J. Pharmacol. Sci*., **93**, 95-105 (2003).
3. C. L. Bethea, N. Z. Lu, C. Gundlah, and J. M. Streicher, *Front. Neuroendocrinol*., **23**, 41-100 (2002).
4. R. B. Gibbs, *Brain Res*., **757**, 10-16 (1997).
5. V. W. Henderson, A. Paganini-Hill, C. K. Emanuel, *et al*., *Arch. Neurol*., **51**, 896-900 (1994).
6. M. Luttgen, E. Elvander, N. Madjid, and S. O. Ogren, *Neuropharmacology*, **48**, 830-852 (2005).
7. M. Ohno and S. Watanabe, *Brain Res*., **736**, 180-188 (1996).
8. A. M. Palmer and S. T. DeKosky, *J. Neural. Transm. Gen. Sect*., **91**, 135-159 (1993).
9. P. Jr. Riekkinen, *Eur. J. Pharmacol*., **262**, 77-90 (1994).
10. J. W. Simpkins, P. S. Green, K. E. Gridley, *et al*., *Am. J. Med*., **103**, 19S-25S (1997).
11. M. Singh, E. M. Meyer, and J. W. Simpkins, *Endocrinology*, **136**, 2320-2324 (1995).
12. W. J. Wilson and J. A. Cook, *Acta Neurobiol. Exp. (Wars.)*, **54**, 377-391 (1994).