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Comparison of the Effect of NT-0409 and Antidementia Drugs on Learning and Memory in Rats with Chronic Cerebral Cholinergic Deficiency

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Systemic oral administration of NT-0409, a new synthetic agonist of AMPA subtype glutamate receptor, to rats with chronic partial AF64A-induced deprivation of cholinergic functions improved their learning in a Morris water maze. NT-0409 is close to memantine by the effect on learning and, in contrast to cholinomimetic arisept, ensures longer retention of the developed habit.

Key Words: *Alzheimer disease; AF64A; memantine; arisept; AMPA receptor agonist; Morris test*

Alzheimer disease (AD) usually develops in advanced age and is characterized by progressive degeneration of primarily acetylcholine neurons and dysfunction of other neurotransmitter systems of the brain. About 10 drugs are now used for the treatment of AD, the majority of these drugs, *e.g.* arisept, produce a pronounced cholinomimetic effect. Only few drugs improve the cognitive functions through other neurotransmitter systems. Akanitol memantine (Merz) acts through glutamate receptors as low-affinity non-competitive NMDA receptor antagonist and AMPA receptor agonist [1]. We previously showed that some isothiuronium derivatives potentiate activity of AMPA glutamic receptors and hence, can be regarded as potential stimulators of cognitive functions [1]. In light of this it was interesting to study the effect of NR-0409, a potent stimulator of AMPA receptors, on learning in animals in a state close to manifest AD.

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In rats AD can be modeled by intracerebroventricular injections of neurotoxin AF64A (1-ethyl-1-[2-hydroxyethyl]aziridinium chloride) [2,4,8,11] inducing selective destruction of acetylcholine synaptic endings followed by neuronal degeneration and decrease in the content of the hippocampal and cortical acetylcholine markers [5,6,11].

Here we studied the effect of systemic administration of a novel preparation NT-0409 on learning in rats with chronic deprivation of cholinergic functions, induced by intracerebroventricular injections of AF64A and compared the effect of this new agent with the effects of the drugs used for the treatment of AD.

MATERIALS AND METHODS

The study was carried out in winter on male Wistar rats from Stolbovaya Breeding Center (180-200 g) after 1-week quarantine. AF64A was prepared from AF64 (Bachem) before injections using Fisher's method [5] and diluted with artificial cerebrospinal fluid (CSF). AF64A (3 nmol in 3 μl CSF) was injected stereotactically into the lateral cerebral ventricles under

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ether narcosis, the controls were injected with 3 μl CSF. One day after AF64A injection the rats were divided into 4 groups. Controls and animals of one experimental group received orally 1% starch suspension (0.1 ml/kg daily). The animals of the other two groups received 2 mg/kg NT-0409, memantine HCl (RBI), and arisept (dopezil HCl, Pfizer; pulverized tablets, 2 mg/kg active substance) suspended in 1% starch. After 10-day treatment the rats were trained to find a submerged round platform (d=10 cm) in a round basin (d=180 cm) according to the standard Morris test paradigm [7,9]. The time of the experiment was then counted starting from day 1 of learning. The training sessions were repeated 2 times a day with a 10-min interval from 2 different start points. The test drugs were administered 1-1.5 h after the session. On day 1 the animals were given 120 sec to find the platform, in subsequent days 60 sec. On days 11-13 the drugs were given, but training sessions were not performed. After day 13 both treatment and learning were discontinued, and on day 26 memory retrieval was tested. The latency of finding the platform was recorded every day.

On days 5, 11, 14, and 26 after the beginning of learning the tracks were videotaped using Behavioral Vision complex and the latency of reaching the platform and track straightness index (ratio of track length to the length of the vector connecting the start point and the platform) were analyzed [10]. The total distance was calculated by the formula:

$$\sqrt{\sum_{i=0}^{N}|R_i|},$$

where N is the number of digitized points of the track and R_i is the distance between the i-th point in the trajectory and the center of the platform.

The results were presented as $M \pm m$ for each parameter. The data were processed statistically (ANOVA) with subsequent comparison using the LSD test.

RESULTS

The Morris test is based on instinctive attempts of the animal to get from water; the gist of the test is that instead of long useless swimming near the basin walls the rat swims with certainty towards a submerged platform (Fig. 1). As a rule, after days 8-10 of learning (2 trials daily) the majority of rats find the platform within 10-15 sec [4,7,9]. After intracerebroventricular injections of AF64A the latency of finding the platform increased [4] and the tracks were more chaotic (Fig. 1) compared to the control.

In our experiments the latency of finding the platform decreased by the end of learning in all groups. On day 11 this parameter in groups AF64A+NT-0409 and AF64A+arisept differed significantly from that in AF64A group (Table 1). On days 11 and 14 the differences in LP between groups AF64A and controls were insignificant (p=0.077 and p=0.065, respectively), but the graphic presentations of the summary trajectories (Fig. 1) looked different. The straightness and summary distance to the platform more reliably reflected the difference in the trained habits of controls and animals receiving AF64A (Table 1).

In rats of all correction groups the search for the platform after learning (days 11, 14) was more directed in comparison with AF64A group (Fig. 1). The differences were significant by all parameters in AF64A+ arisept group, while in other groups the differences were significant for only some parameters (Table 1). Arisept considerably shortened the latency of finding the platform and straightened the trajectory as early as on day 5 of learning. A 2-day interval after learning (against the background of continued treatment) slightly increased the latency of finding the platform in all rats, but in rats treated with arisept or NT-0409 this parameter was significantly shorter than in rats receiving AF64A alone (Table 1).

Twelve days after termination of treatment, the results in the AF64A+arisept group were close to those in the AF64A group, while the search parameters in AF64A+NT-0409 and AF64A+memantine groups were better than in AF64A group (Table 1).

Hence, the symptomatic effect of cholinomimetic arisept rapidly manifests during treatment and rapidly disappeared after discontinuation of the drug therapy. The effects of memantine and NT-0409 manifested later than that of arisept, but persisted longer after discontinuation of the treatment. In our experiments the drugs were injected 1.0-1.5 h after learning sessions, therefore they could affect memory consolidation. However, 10-day administration of the test drugs after injection of AF64A preceding learning could modulate the degenerative-regenerative processes caused by the cholinotoxin, and therefore we cannot make unambiguous conclusions about positive effects of the studied drugs on the consolidation process.

Experimental conditions simulated a clinical situation occurring in patients with AD. The therapy in these patients is directed toward inhibition of degenerative processes and improvement of cognitive functions. The effect of cholinomimetic arisept observed in our experiments was adequate to our model, but was symptomatic. The more prolonged effect of other test drugs can be realized through glutamate receptors and affect the compensatory and/or degeneration-regeneration processes. NT-0409 (AMPA glutamate receptor agonist) in our experiment had a positive impact on learning and long-term retention of the habit

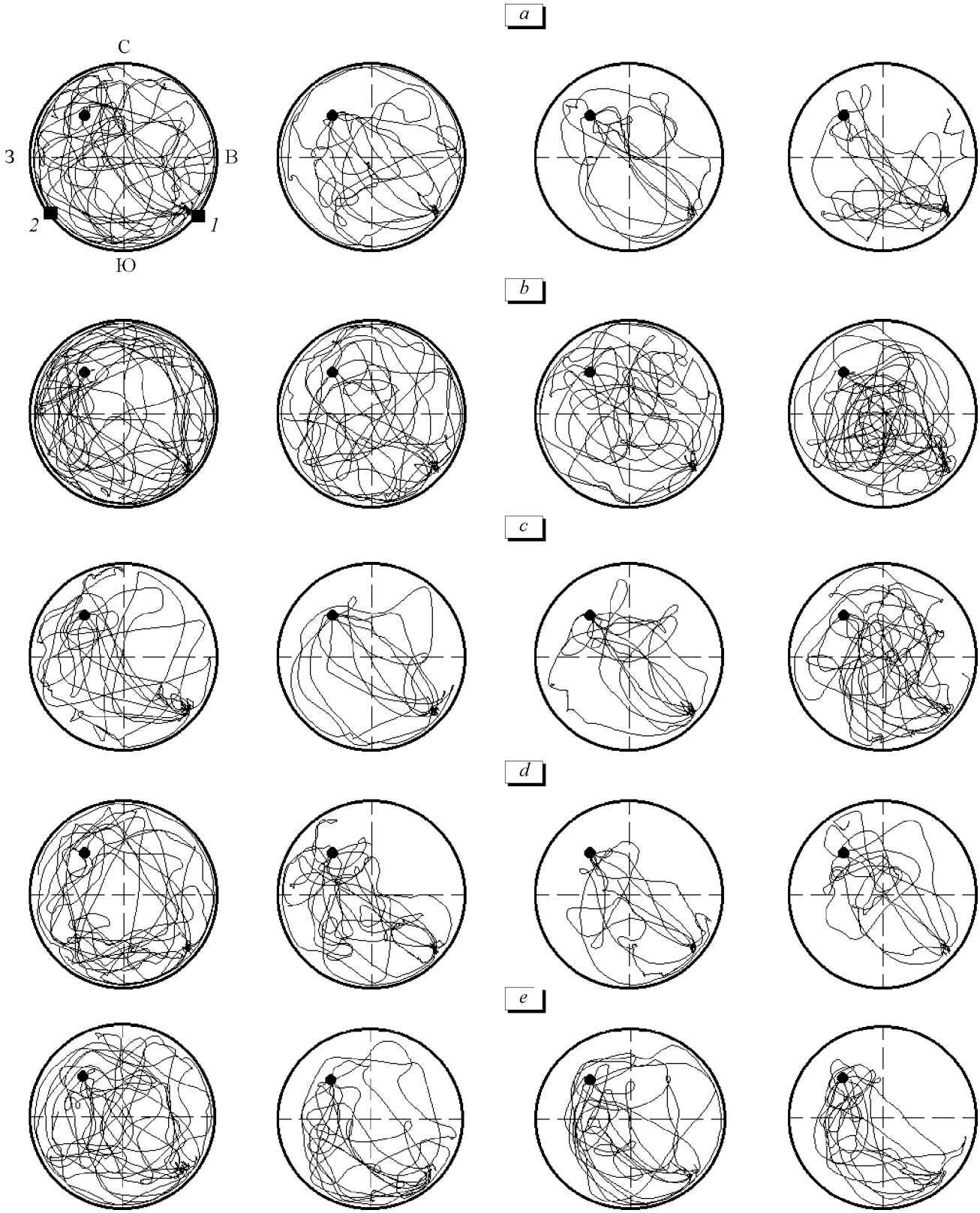

Fig. 1. Effect of intracerebroventricular injection of AF64A on rat swimming trajectory in Morris water maze. *a*) control; *b*) AF64A; *c*) AF64A+arisept; *d*) AF64A+memantine; *e*) AF64A+NT-0409. The circle shows the position of the platform. *1*, *2*: starting points. From left to right: days 5, 11 (learning), 14 (1st retrieval test), and 26 (2nd retrieval test).

TABLE 1. Effects of Arisept, Memantine, and NT-0409 on Rat Learning and Performance in Morris Water Maze after Intracerebroventricular Injection of AF64A (% of Control; *M*±*m*)

Days, parameters		Control (*n*=10)	AF64A (*n*=10)	AF64A+arisept (*n*=10)	AF64A+memantine (*n*=8-9)	AF64A+NT-0409 (*n*=10)
5	latency of finding the platform	100.0±12.9	104.5±11.3	65.5±14.8**	74.7±17.5	82.9±14.7
	straightness index	100.3±17.8	115.6±19.8	51.9±8.6**	90.4±23.7	85.0±17.6
	total distance to the platform	100.0±8.5	109.3±8.7	80.2±11.8**	84.8±13.9	92.6±11.9
11	latency of finding the platform	100.0±32.1	163.7±28.9	63.4±9.9*	120.3±32.7	91.5±15.8**
	straightness index	100.3±32.0	150.4±34.9	63.7±15.1**	101.8±15.8	94.0±17.0
	total distance to the platform	100.0±18.8**	143.5±16.4	91.6±9.1**	109.8±17.7	101.1±11.4**
14	latency of finding the platform	100.0±34.1	173.1±27.3	88.8±14.7**	105.4±32.8	96.1±21.8**
	straightness index	100.0±17.7**	196.8±56.5	95.7±11.8**	91.4±11.3**	164.0±40.4
	total distance to the platform	100.0±17.3*	157.5±13.7	105.1±11.9**	116.9±21.6	100.1±12.2*
26	latency of finding the platform	100.0±23.8	135.8±17.9	136.2±29.2	91.8±30.8	102.8±25.7
	straightness index	100.0±21.3**	231.0±65.0	187.3±44.7	106.6±23.9**	128.5±30.8
	total distance to the platform	100.0±16.2	122.7±8.2	112.2±16.5	81.5±15.5**	92.3±12.5**

Note. *p<0.01, **p<0.05 compared to AF64A.

in rats with partial chronic deprivation of cholinergic functions.

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