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Dimebon Improves Learning in Animals with Experimental Alzheimer's Disease

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Systemic administration of antihistamine drug dimebon improves active avoidance conditioning in rats with chronic partial deprivation of cerebral cholinergic functions caused by intracerebroventricular injections of AF64A. The effects of dimebon on learning are similar to those of tacrine used in the treatment of Alzheimer's disease.

Key words: *dimebon; H_1 receptor inhibitor; tacrine; AF64A; avoidance conditioning; rats*

Alzheimer's disease (AD) occurring primarily in elderly persons is characterized by neurodegenerative processes in the brain reducing cerebral content of acetylcholine (AC) and its markers [6,12]. Apart from new drugs for AD treatment compensating for AC deficiency or interacting with its receptors or enzymes responsible for its synthesis and degradation, compounds whose effects are mediated by other mechanisms have recently been tested. Pilot clinical studies showed that Russian-made antihistamine preparation dimebon (3,6-dimethyl-9-(2-methyl-pyridyl-5)-ethyl-1,2,3,4-tetrahydro-γ-carboline dihydrochloride) [3] improves cognitive functions in AD patients [2]. The role of histamine, antagonists, and agonists of three types of histamine receptors in learning and memory processes is now extensively studied. Some data are controversial, which may be due to differences in the experimental design and especially in modeling of the disease. 1-Ethyl-1-(2-hydroxyethyl) aziridinium (AF64A), a neurotoxic analogue of AC, causes neurodegenerative disorders similar by their transmitter specificity and localization of brain lesions to those characteristic of AD [6,12]. Administration of AF64A into the lateral cerebral ventricles is currently used as an adequate animal model of AD. Being a false-transmitter, AF64A enters neurons via the high-affinity uptake mechanism and irreversibly inhibits acetylcholinesterase, choline acetyltransferase, choline release and reuptake, and axoplasmic transport. AF64A binds DNA, thereby causing death of cholinergic neurons. Specifically, AF64A considerably decreases the content of choline acetyltransferase RNA in neurons of the septohippocampal tract [9]. One or two days after administration of 1-3 nmol of AF64A into the lateral cerebral ventricles, the content of AC in the cerebral cortex and hippocampus decreases and remains low for about 6 months [6,12]. Our previous studies showed that AF64A impairs acquisition of the two-way active avoidance reaction (TWAA) in rats, whereas tacrine (9-amino-1,2,3,4-tetrahydroacridine), a drug patented in the USA (trade name Cognex [5]) for the treatment of AD, can compensate these learning disorders [14]. Active avoidance paradigm allows to neglect sedative effect characteristic of some histamine receptor antagonists, including dimebon [3]. Studies of the effects of dimebon on learning in animals with chronic depression of cerebral cholinergic functions help to understand the mechanisms of the effects of dimebon on

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cognitive functions and facilitate screening of drugs against AD among its analogues.

The aim of the present study was to compare systemic effects of dimebon and tacrine (as a reference drug) on acquisition of TWAA in rats with experimental AD caused by AF64A.

MATERIALS AND METHODS

Male Wistar rats weighing 280-350 g were purchased from Stolbovaya nursery and kept under standard vivarium conditions. AF64A was freshly prepared from AF64 (Bachem) by the method described by Fisher [10] and dissolved with artificial cerebrospinal fluid (CSF). AF64A (3 nmol in 3 μl CSF) was injected into the lateral cerebral ventricles through a stereotaxically implanted cannula under ether anesthesia. Control rats received 3 μl CSF. The stereotaxic coordinates were [3]: AP=-0.8 mm, L=±1.5 mm, and H=3.5 mm. One day after AF64A administration, the rats were divided into three groups assigned to receive tacrine (Sigma), dimebon (Organica), or 0.1 ml 0.9% NaCl (groups 1, 2, and 3, respectively). Tacrine and dimebon were injected intraperitoneally in a dose of 1 mg/kg body weight in 0.1 ml 0.9% NaCl. The rats were trained active avoidance task in a shuttle box with a guillotine-type door as described elsewhere [1]. Electric current (1 mA) delivered to the floor of one compartment of the shuttle box served as an unconditioned stimulus. Conditioned light stimulus (lamp in the same part of the shuttle-box) was presented 5 sec before electrical stimulation and simultaneously with door opening. Transition to the dark compartment after light stimulus before electrical shock served as a criterion of successful TWAA acquisition. The training session consisted of 35 presentations of the conditioned stimulus for each rat, and retention of memory traces was tested on the next day by the same method. The latency of TWAA (the time from light stimulation to transition) and the number of conditioned runs were recorded. The number of rats meeting the acquisition criterion (8 consecutive correct responses) was determined for each group. In addition, the mean number of runs per series of 5 presentations was recorded for 15 last runs on day 1 ((training) and for the first 15 runs on day 2. Data of two independent experimental series (36-40 rats per each) were analyzed statistically by one-way ANOVA followed by Newman—Keuls group comparison test.

RESULTS

In our experiments, AF64A was administered in a dose of 3 nmol per ventricle because numerous studies on rats showed that 1-3 nmol/ventricle AF64A reduced the content of AC in the cerebral cortex and hippocampus by 22-44% on days 1-2 after administration [12,13]. AF64A degrades *in vivo* within 24 h [12]; therefore, test drugs were administered one day after AF64A administration in order to avoid its interaction with the test drugs. The daily doses were chosen on the basis of our previous studies demonstrating that tacrine in a dose of 1 μg/kg improved TWAA performance [14].

On days 12-14 after AF64A administration the number of conditioned avoidance responses decreased by 34.4±7.4 and 30.3±7.4% on days 1 and 2, respectively, compared to the control (Table 1). In rats receiving dimebon and tacrine for 12-14 days after AF64A administration, TWAA acquisition practically did not differ from that in the control group (Table 1). The most pronounced differences between the groups treated with dimebon and tacrine were revealed, when the acquisition criterion was taken into account. Most (60.0-75.6%) rats of group 1 displayed certain learning abilities, but were unable to perform a series of 8 correct TWAA responses, whereas in test groups 2 and 3 this parameter was close to the control. Some rats, which attained the acquisition criterion on day 1, reproduced this result in the second test, which indicated sufficient memory retention.

Thus, systemic administration of dimebon and tacrine restored TWAA performance in AF64A-injected rats practically to the control level, which suggested that these drugs activated some compensatory mechanisms and/or prevented neurodegeneration. Improvement of learning in animals with impaired cognitive functions resulting from disturbances in the cen-

TABLE 1. Effects of Dimebon and Tacrine on Learning in Rats with Partial Deprivation of Cholinergic Functions Caused by Administration of AF64A into Lateral Cerebral Ventricles (% of Correct Responses, *M*±*m*)

Day of experiment	Control	AF64A		
		+0.9% NaCl	+dimebon	+tacrine
1st	100±5*	65.6±7.4	88.0±5.7**	85.4±6.7**
2nd	100.0±6.1**	69.7±7.4	95.9±8.3**	95.6±7.2**

Note: *$p<0.005$, **$p<0.05$ compared to AF64A+0.9% NaCl.

TABLE 2. Effects of Dimebon and Tacrine on the Number of Rats Performing a Series of 8 Correct Responses (% of Control)

Day of experiment	CSF+0.9% NaCl (control, n=18)	AF64A		
		+0.9% NaCl (n=19)	+dimebon (n=19)	+tacrine (n=19)
1st	10 (100)	4 (40)	10 (100)	9 (90)
2nd	11 (100)	4 (36.4)	10 (91)	10 (91)

tral nervous system of various origin or in animals with scopolamine-induced amnesia treated with tacrine was described by other researchers [7]. We previously observed this effect of tacrine in animals treated with high doses of AF64A [14]. In this work we used tacrine as a reference drug because of its ability to improve TWAA acquisition. The cognitive effects of tacrine have been initially explained by its anticholinesterase activity; further investigations showed that this drug exhibits a broad spectrum of biochemical and physiological effects [7]. However, the mechanisms underlying the positive effect of tacrine on cognitive functions under pathological conditions are poorly studied. It also remains unclear whether or not tacrine and dimebon act through the same mechanisms. However, indirect effects of dimebon on the cholinergic system cannot be excluded because its interaction with H_1 receptors can elevate the content of AC in the brain. It was demonstrated that systemic administration of H_1 receptor antagonist chloropheniramine facilitates learning in aged rats to a level characteristic of young animals [13]; this effect of chloropheniramine was explained by dose-dependent (1.5-3-fold) increases in the cortical and hippocampal AC [8].

In this work, we showed for the first time a positive effect of dimebon on TWAA performance after chronic partial deprivation of cerebral cholinergic functions. Our findings provide the basis for further studies of the mechanisms of its effects on learning and memory under pathological conditions.

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