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Effects of Tacrine on Deficits in Active Avoidance Performance Induced by AF64A in Rats

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ABSTRACT

Effects of tacrine (1,2,3,4-tetrahydro-9-aminoacridine) on memory deficits in rats treated with ethylcholine aziridinium ion (AF64A) were studied using active avoidance test in the two-way shuttle box. Neurotoxin AF64A injected at a dose of 6 nmol (icv, bilaterally) causes nonspecific tissue damage in hippocampal fields CA2 and CA3. Two weeks after treatment with 6 nmol, AF64A active avoidance performance of toxin-treated rats was significantly deteriorated compared to vehicle-treated animals estimated in learning test (68 ± 3.5 and 83 ± 3.2% of correct responses, respectively; $p < 0.01$) and in retention test (53 ± 5 and 76 ± 3.6%, respectively; $p < 0.01$). Under these conditions, chronic treatment with tacrine at a daily dose of 1 mg/kg for 12–14 d reverses the effect of AF64A on the active avoidance performance both in learning (78 ± 3.2%) and retention (72 ± 4%) tests. It is supposed that behavioral effects of tacrine considerably depend on a severity of neurodegeneration in the hippocampus.

Index Entries: Alzheimer disease; chemically induced; neurotoxin AF64A; active avoidance test; tacrine; rats.

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INTRODUCTION

Senile dementia of Alzheimer type, or Alzheimer disease (AD), is a neurodegenerative disorder with impairment of cognitive function of brain and memory deficit. A major neurochemical feature of AD is a loss of cholinergic markers, associated with the neuronal atrophy. It should be noted that only a few drugs have been approved for AD treatment up to now (Prous, 1995). Tacrine hydrochloride (1,2,3,4-Tetrahydro-9-aminoacridine, Cognex™) was proposed in 1993 in the United States as an acetylcholinestease inhibitor for the treatment of both mild and moderate forms of AD. However, quite complex pharmacological profile of the compound have been revealed recently (Freeman and Dawson, 1991; Cacabelos et al., 1994). Tacrine demonstrates significant properties of cognition enhancement with pronounced therapeutical effects observed in 25–56% of the cases. The variability of therapeutic response of AD patients observed during the tacrine treatment might be owing to different subtypes of AD and a degree of neurodegeneration in different regions of brain (Cacabelos et al., 1994).

In order to explore the morphological, biochemical, and molecular correlations of brain functions in the course of AD, several toxin-induced AD models were developed (Woodruff and Baisden, 1994; Yokel R. A., 1994). One of the most promising models accentuates the role of neurotoxic choline analog, namely ethylcholine aziridinium ion (AF64A) (Fisher and Hanin, 1980; Hanin, 1996). As was shown by histochemical and electronomicroscopic studies of brain, icv injection of 0.5–3 nmol AF64A results in specific degeneration of cholinergic axon terminals in hippocampus, whereas higher doses additionally produce a dose-dependent tissue damage, especially in the fields of CA2 and CA3 (Kasa et al., 1986; Hanin, 1996). The treatment with AF64A was demonstrated to produce dose-dependent decrease in learning and memory functions of rats (Walsh and Hanin, 1986; Walsh and Opello, 1994). It was revealed as well that some agents with different mechanisms of action can ameliorate AF64A-induced memory deficits (Fisher et al., 1989; Abe et al., 1994; Murai et al., 1994; Gozes et al., 1996).

The goal of the present work was to investigate the effects of tacrine on memory and learning impairments in experimental animals in active avoidance tests and to analyze its possible neuroprotective properties using AF64A model.

MATERIALS AND METHODS

Male Wistar rats of 12–16 wk age and 280–450 g weight purchased from the vivarium "Stolbovaja" were used in the experiments. Two weeks before the experiment and during the tests, the rats were housed in individual cages at a 12-h light–dark cycle and had a laboratory diet.

The precursor of AF64A, namely acetylcholine mustard hydrochloride, was synthesized in the Institute of Physiologically Active Substances,

Russian Academy of Sciences, and identified by nuclear magnetic resonance. A fresh solution of AF64A was prepared each day before the injection and then kept on ice (Fisher and Hanin, 1986). The precursor was allowed to cyclize for 30 min at the media pH equal to 11.3 and the room temperature. The value of pH was then decreased to 7.4 using HCl, and the solution obtained was diluted to AF64A concentration of 2 nmol/μL using artificial cerebrospinal fluid (CSF). CSF having pH equal to 7.4 was used for injections into the ventricles of vehicle-treated rats as well.

The rats were anesthetized with ether and placed into a stereotaxic frame. The skull surface being exposed, a small hole was made in it using a hand drill. The coordinates for injection sites were selected using the rat brain atlas (Pellegrino et al., 1981), namely, posterior –0.8 mm for the bregma, lateral ±1.5 mm, and ventral 3.5 mm. Then 3 μL of AF64A solution (6 nmol/p ventricle) or CSF was injected through cannulas inserted into the holes in the skull at a rate of approximately 1.5 μL/min.

After surgery, the rats were given a recovery period of 12–14 d before the behavioral experiment. The day after the surgery and during the entire recovery period, the rats from a vehicle-treated group and from one of the two groups treated with AF64A were daily (once a day) injected with 0.9% NaCl saline (1 mL/kg). For the animals from another AF64A-injected group, saline with tacrine was used. Tacrine (Sigma) was dissolved in 0.9% NaCl saline (1 mg/mL) and administered ip at a dose of 1 mg/kg. Each group of the rats consisted of 6–8 animals, 69 rats being used in three independent experiments.

Two weeks after the injection of AF64A or CSF, active avoidance training was performed using a two-chamber shuttle box according to the procedure described earlier (Bures et al., 1983). Each chamber was 30 cm long and 20 cm wide, the chambers being separated by a "guillotine" door (7 × 7 cm). The shuttle box had a grid floor made of steel rods located at a distance of 1 cm from each other. A 12-W lightbulb was mounted on the ceiling of each chamber. Before training, the animals were given a 5-min familiarization period in the darkened shuttle box with the central door opened. The light used as a conditioned stimulus was followed after 5 s by the unconditioned one, namely a 1-mA shock fed to the grid of the lit chamber. The door was opened during the action of both stimuli. After the rat avoided the shock running into the other chamber, the door was closed and a new trial sequence was started. The intertrial interval was 30–60 s. During the experiments, the time required for the rats to avoid the shock (response latency) was measured. The avoidance during the conditioned stimulus was considered as conditioned avoidance response (CAR).

Calculation and Statistical Analysis

Training procedure consisted of 35 successive trials. Data from last 15 trials (learning test) were collected and analyzed in five-trial blocks as a percentage of CAR summarized over each block. Then these data were

presented as the mean percentage of CAR in three blocks. Twenty-five further trials (retention test) were performed 24 h after the learning test. In this case, data were analyzed as the mean percentage of CAR in first two blocks of five trials. Statistical significance was tested using unpaired Student's *t*-test. The value of $p < 0.05$ was considered as an index of statistical significance.

Histology

Behavioral tests having been completed, animals were anesthetized with pentobarbital. The brains were fixed in 10% formaline solution. Frozen frontal sections of a thickness of 20–40 μm were cut using a microtome, and then each fifth sample was mounted on gelatin-coated slides. The brain samples were stained with toluidine blue for microscopic analysis.

RESULTS

In the first 20 trials of active avoidance test, AF64A-injected rats generally demonstrated a longer time of avoiding the shock than vehicle-treated rats (6.2 ± 0.29 vs 5.6 ± 0.26 s, $p < 0.05$). Although this negative effect of AF64A injection on avoidance performance has been revealed, we observed no statistically significant difference of CAR between groups studied in the first series of the trials. The results obtained for 15 further trails in the training test are presented in Fig. 1. In this case, the vehicle-treated control animals demonstrated 83 ± 3.2% of correct CAR, whereas active avoidance performance of the AF64A-injected group was at the level of 68 ± 3.4% ($p < 0.01$). Thus, these results indicate that icv injection of AF64A produces significant learning deficit. Since the learning ability represents CAR more correctly than the response latency, the last trials (from 21–35) were the most informative ones in our experiments.

The results of the retention test carried out 24 h after the training are presented in Fig. 2. The animals injected with CSF exhibited excellence avoidance performance in the first series of 10 trials (76 ± 3.6%). However, AF64A-injected group performed only 53 ± 5% of correct responses in these trials; i.e., this group exhibited significant memory deficit compared to the control rats ($p < 0.01$). Despite this memory impairment revealed in the first trials, AF64A-injected rats were still able to perform the learning in the series of 15 further trials in which both the groups demonstrated similar levels of active avoidance performance. Thus, AF64A-injected rats had the pronounced memory impairment, which was statistically significant within first 10 trials of the retention test.

AF64A-injected rats treated with tacrine during the period of 12–14 d before being tested exhibited avoidance performance similar to that of control animals (78 ± 3.2%; Fig. 1). In addition, the CAR percentage of this group was significantly higher in comparison to another AF64A-

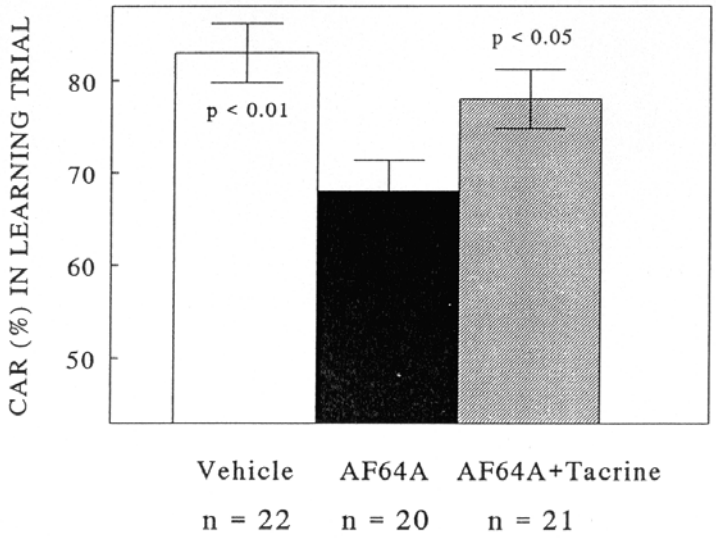

Fig. 1. Shuttle-box avoidance performance of vehicle-treated, AF64A-injected rats, and AF64A-injected rats treated with tacrine (1 mg/kg, once daily, 12–14 d, ip). Data are presented as the mean ± SEM percentage of correct responses in three five-trial blocks, which followed 20 acquisition trials. Values of p illustrate that there is a significant difference in avoidance performance vs AF64A-injected group (Student's t-test; n is the number of rats in each group). The results in this figure derive from the data obtained in three independent experiments.

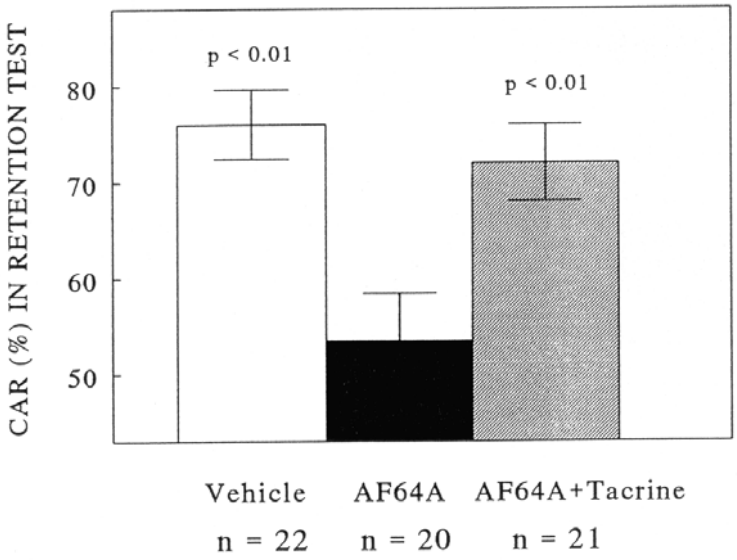

Fig. 2. Shuttle-box avoidance performance of vehicle-treated, AF64A-injected rats, and AF64A-injected rats treated with tacrine (1 mg/kg, once daily, 12–14 d, ip). Two blocks of five trials were given 24 h after learning trial. Data are presented as the mean ± SEM percentage of correct responses. Values of p illustrate that there is a significant difference in avoidance performance vs AF64A-injected group (Student's t-test, n is the number of rats in each group). The results in this figure derive from data obtained in three independent experiments.

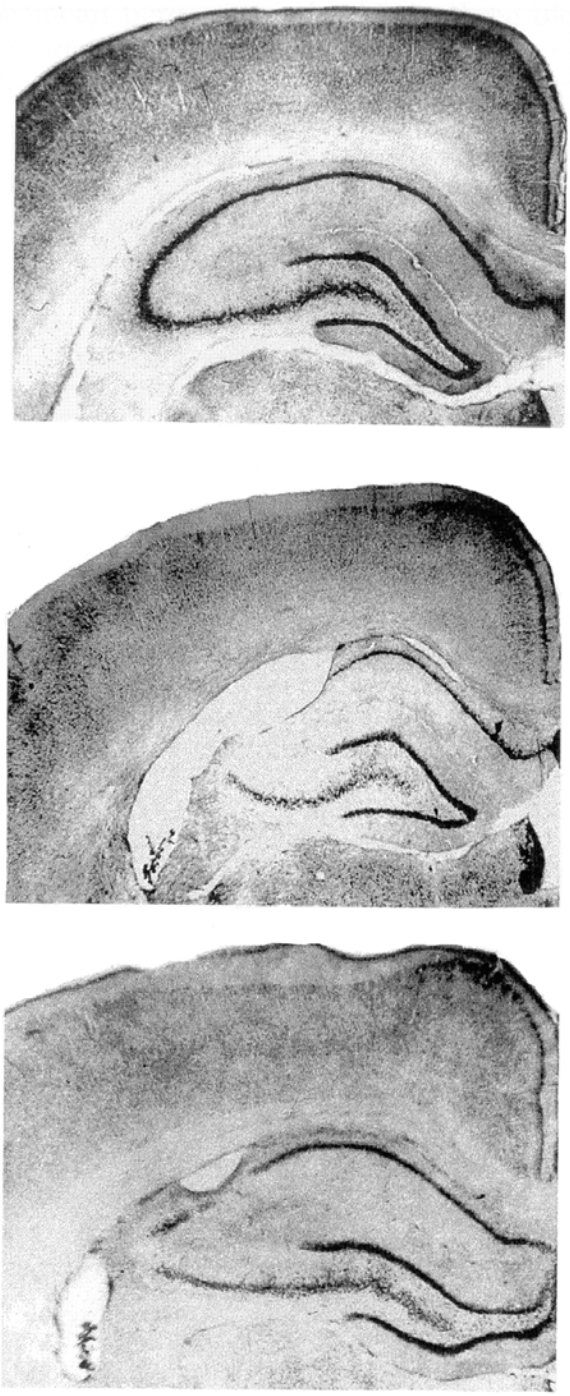

Fig. 3. The effect of icv injection of AF64A on the hippocampal formations. Light microscopic appearance of the Nissle-stained frontal sections through the brain of rats. The rats were analyzed 18 d after icv injection. **(A)** Control, 3 µL CSF icv; the rat was daily (first 2 wk) injected ip with 0.9% NaCl. **(B)** 6 nmol/3 µL AF64A icv; the rat was daily (first 2 wk) injected ip with 0.9% NaCl. Hippocampus was reduced in CA2 and CA3 region. It is the sample with the maximum necrotic area. **(C)** 6 nmol/3 µL AF64A icv; the rat was daily (first 2 wk) injected ip with 1 mg/kg of tacrine in 0.9% NaCl. The CA2 and CA3 regions of hippocampus are reduced partly, and some pyramidal neurons have disappeared.

injected group, which was not treated with tacrine ($p < 0.05$). In the retention test (Fig. 2), the avoidance performance of the tacrine-treated group was also comparable to controls (72 ± 4%) and significantly higher than that of observed for another AF64A-injected group ($p < 0.01$). The results demonstrated that chronic administration of tacrine significantly decreases AF64A-induced learning and memory impairments.

Injection of 6 nmol AF64A (icv) was found during the histological analyses to produce neuronal degeneration in the most sensitive parts (CA2 and CA3) of the dorsal hippocampus. On the average, size of degenerative area fluctuated from 0.1 up to 2 mm in diameter both in AF64A-injected rats and in AF64A-injected rats treated with tacrine. In some hippocampii, we observed the loss of 30–50 pyramidal neurons without degeneration of other cells. In five brains from AF64A-injected rats, the dorsal areas of hippocampus in regions CA2 and CA3 were reduced and maximal necrotic area had a diameter of about 1–2 mm (Fig. 3B). Four hippocampii of AF64A-injected rats treated with tacrine had the same size of necrotic areas, which were partially filled by glial cells in these cases (Fig. 3C). We did not detect any visible gliosis in hippocampii with small areas of neuronal degeneration in both groups of AF64A-injected rats. Moreover, histological analyses demonstrated that both AF64A-injected groups had some neural damage in the lateral septum. Brains in all the AF64A-injected rats exhibited some ventricular dilatation.

DISCUSSION

The effects of AF64A on the cognitive functions of brain were studied using different behavioral models. It was demonstrated that icv injection of AF64A produces significant learning and memory deficits in the experiments with R- or T-mazes, Morris water-maze, and passive avoidance test (Nakahara et al., 1988; Fisher et al., 1989; Gower et al., 1989; Hashimoto et al., 1991; Walsh and Opello, 1994). There are contradictory data in the literature concerning the AF64A-induced learning and memory deficits studied in the active avoidance test. In the test of one-way active avoidance, icv injection of 2 nmol AF64A induced impairment of learning and memory (Ogura et al., 1987). On the other hand, icv injection of 3 nmol AF64A did not affect the active avoidance response studied in the two-way shuttle box (Nakahara et al., 1988). Probably, this variance reflects differences in the technique of the experiments and/or in the chamber constructions (Bailey et al., 1986). In addition, it has been shown that slight necrosis of hippocampus was observed at the injection of the neurotoxin at a dose of 1 and 3 nmol/ventricle (Gower et al., 1989). At low doses, the lesion effect of AF64A can depend on the variety of such factors as purity of the starting compound, concentration, injection rate and volume, and site of administration (Chrobak et al., 1989; Hanin,

1996). In our previous investigation, bilateral injection of AF64A at a dose of 3 nmol per ventricle produced small, but statistically significant learning and memory deficits (data are not presented). In the present work, AF64A injected at a dose of 6 nmol/ventricle was shown to cause (1) a remarkable disappearance of pyramidal neurons and the neighboring glial cells in the hippocampal fields CA2 and CA3 (Fig. 3B), and (2) statistically significant learning and memory deficits in the two-way shuttle box. We suppose the significant deficits observed in the active avoidance test to be related to a pronounced cell loss in hippocampus induced by high doses of AF64A. These results are quite consistent with those obtained by Bailey and his colleagues (1986), which found the intrahippocampal injection of 5 nmol AF64A to lead to a nonspecific tissue damage near the site of application and considerably reduce active avoidance response studied in the two-way shuttle box.

It is necessary to emphasize that, during 24 h, AF64A is quite inactive in vivo (Hanin, 1996). Therefore, in our experiment, tacrine treatment was initiated 24 h after icv injection of AF64A. There are no data concerning duration of the neurodegenerative process in hippocampii after icv injection of AF64A. As was found elsewhere, the neurochemical effect of AF64A administration does not appear until 2–3 d, and depletion of ChAT and AChE level increases significantly in 10–14 d (Hanin, 1996). Therefore, AF64A can be supposed to initiate only the process of degeneration, with takes 2–3 d and longer.

It is known that effects of tacrine depend on the place of brain injury, dose, and memory task (Riekkinen et al., 1991, 1992; Riekkinen and Riekkinen, 1995). There are few data reports about effects of tacrine on AF64A-induced learning and memory deficit studied in active avoidance tests. The dose of 1 mg/kg being generally used in AD therapy, we applied daily chronic treatment with tacrine at this dose. Similar doses of tacrine (1–2.5 mg/kg) produce remarkable inhibition of AChE (22–44%) in hippocampus, cortex, and cerebellum, and demonstrate maximally improved retention performance in mice (Sherman and Messamore, 1989). The effect of chronic administration of tacrine on the AF64A-induced memory impairment in mice was studied in a delayed nonmatching to sample task (Murai et al., 1994). It was found that tacrine did not improve memory deficits at any doses tested (0.3–3.0 mg/kg, once daily for 11 d) and did not reverse the AF64A-induced hippocampal acetylcholine depletion. In another report, tacrine partially reversing the effect of anoxia or AF64A on the passive avoidance performance did not change the active avoidance response in rat pups (Speiser et al., 1989). In both cases, the neurotoxin was administered at low doses, which did not produce a remarkable tissue damage in hippocampus. In contrast to these data, we have found chronic treatment with tacrine to reverse significantly the effect of the injection of 6 nmol/ventricle AF64A on the active avoidance performance in both the learning and retention tests (Figs. 1 and 2). The similar effect of tacrine was observed in the experi-

ments with basal forebrain lesioned rats (Nabeshima et al., 1991) and rats lesioned with excitotoxins (Hodges et al., 1990). These results led us to the suggestion that mnemonic effects of tacrine are more evident at significant brain lesions, such as necrotic tissue damage. Tacrine did not prevent the loss of pyramidal neurons in our experiments, but protected against AF64A-induced retardation of learning and memory. The hippocampal damage in tacrine-treated rats seems to be less pronounced owing to greater gliosis (Fig. 3C). These results led us to a conclusion that the behavioral effect of tacrine is owing to the activation of the compensatory mechanisms in the remaining neurons and glial cells.

Thus, the results obtained demonstrate that icv injection of AF64A at a dose of 6 nmol/ventricle induces a lysis of nervous tissue in CA2 and CA3 fields of hippocampus, and significant impairment of learning and memory processes in the two-way shuttle box active avoidance test. Under these conditions, the chronic treatment with tacrine leads to obvious decrease of the effect of AF64A on cognitive functions. Mnemonic effects of tacrine seem to depend considerably on a severity of neurodegeneration in the hippocampus.

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