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Impairments of Learning and Memory Following Intracerebroventricular Administration of AF64A in Rats

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Three types of learning and memory tests (Morris water maze, active and passive avoidance) were performed in rats following intracerebroventricular infusion of ethylcholine aziridium (AF64A). In Morris water maze, AF64A-treated rats showed the delayed latencies to find the platform from 6th day after the infusion. In pretrained rats, AF64A caused the significant delay of latency at 7th day, but not 8th day. In the active avoidance for the pretrained rats, the escape latency was significantly delayed in AF64A-treatment. The percentages of avoidance in AF64A-treated rats were less increased than those in the control. Especially, the percentage of no response in the AF64A-treated rats was markedly increased in the first half trials. In the passive avoidance, AF64A-treated rats shortened the latency 1.5 h after the electronic shock, but not 24 h. AF64A also caused the pretrained rats to shorten the latency 7th day after the infusion, but not 8th day. These results indicate that AF64A might impair the learning and memory. However, these results indicate that the disturbed memory by AF64A might rapidly recover after the first retrain. Furthermore, these results suggest that AF64A may be a useful agent for the animal model of learning for spatial cognition.

Key words: Ethylcholine aziridium, Morris water maze, Active avoidance, Passive avoidance

INTRODUCTION

Alzheimer's disease is progressive disorder characterized by cognitive and behavior dysfunction. The essential symptoms of dementia are impairments of memory, judgement and abstract thinking (Sunderland *et al.*, 1997). Although the fundamental pathophysiology of Alzheimer's disease remains poorly understood, the pathologic causes are loss of neurons and neuronal connections in the brain (Bartus *et al.*, 1982) and the degeneration of subcortical ascending systems with neuronal losses in multiple neuronal systems (Jellinger, 1997). Neurochemical studies on biopsy and autopsy brain from patients with senile dementia have demonstrated large reductions in activity of the presynaptic marker enzyme choline *O*-acetyltransferase (ChAT) in the cortex and hippocampus (Cassidy *et al.*, 1994).

A lot of chemical agents, the brain trauma and the specific brain lesions are reported for the inductions of Alzheimer' disease and amnesia (Smith, 1988). Ethylcholine aziridinium ion (AF64A) is known to be a selective, irreversible neurotoxin. The infusion of AF64A into either hippocampus or lateral ventricle induced defects of cholinergic nervous system, such as choline uptake, significant reduction of ChAT and AChE activities, reduction of acetylcholine release, and the destruction of the presynaptic terminals in cholinergic nervous systems (Fisher *et al.*, 1982; Ransmayr *et al.*, 1992). And this toxin has been proposed as a tool for studying the functional role of brain cholinergic system and also for the development of animal models of Alzheimer's disease and senile dementia of the Alzheimer type (Fisher *et al.*, 1982; Hanin *et al.*, 1992).

Memory is a complex process consisting of at least four different stages, acquisition, consolidation, retention and retrieval. Several behavioral tests are developed for learning and memory (Vanderwolf and Cain, 1994). These tests are mainly composed of maze- and avoid-

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tests. Although the adequacy of behavioral tests of memory in each test is argued, it is known that avoid-tests are based on the instinctive of the animal and maze-tests are on the intrinsic circuitry, acquired circuitry, or both together (Vanderwolf and Cain, 1994). The AF64A-treated animals have been reported to be impaired in the motivation, learning and memory (Chrobak *et al.*, 1988; Lim *et al.*, 1995; Mannisto *et al.*, 1994). However, the acquisition of T-maze task and a passive avoidance as well as the total responses of active avoidance tasks were reported to be unaffected by the AF64A treatment (Chrobak *et al.*, 1988; Nakahara *et al.*, 1988). Thus, it is still unclear that AF64A-induced behavioral changes are concerned with any process in learning and memory. To examine the usefulness of AF64A for the amnestic animal model, it is necessary to perform systematically in the various behavioral tests and determine a detailed behavioral analysis in spatial behavior of the morris water maze task and in nonspatial behavior tests of both avoidance systems.

Therefore the present study was designed to determine which cognitive impairment was primarily involved in the AF64A-induced impairments of the learning and memory.

MATERIALS AND METHOD

Animals and materials

Male Sprague-Dawley rats weighing 250~300 g were housed at 21 ± 1°C, 50~60% relative humidity on a 12 h light/12 h dark schedule. Animals were freely accessible to food and water. Acetylcholine mustard-HCl was purchased from Research Biochemical Inc. (Wayland, MA, USA). Ethylcholine aziridinium (AF64A) is synthesized by the method reported by Mantione *et al.* (1983).

Animal treatment and experimental protocol

For the administration of AF64A, rats were anaesthetized with Equithsin and mounted in a David Kopf stereotaxic apparatus. The skull was exposed and a guide cannula was implanted according to Paxino and Watson (1986) through the dural surface into the lateral ventricle with respect to bregma at the following coordinates (in mm): A -0.8, L ±1.4, V -4.4. Skull screws and dental cement were used for fixation of guide cannula. A stainless steel obturator was inserted into the guide cannula. Penicillin 30,000 I.U. was administered to protect from infection after surgery. Before the infusion of AF64A, the rats were allowed to recover from surgery for 3 days, housed singly in their cages. AF64A was infused in both ventricles with the rate of 0.5 μl/min (3 nmole/each) at three days after the surgery. The dose of AF64A was chosen according to the report of Meana *et al.* (1992). The control groups were infused with the artificial cerebrospinal fluid. For the learning tests, behavioral tests were performed from 3 to 8 days (for water maze test) and at 7 days (for the passive avoidance test) after the infusion of AF64A. For the retrieval tests, rats were pretrained before the surgery and then behavioral tests were performed at 7 and 8 days after the infusion of AF64A.

Morris water maze test

A circular water tank (140 cm in diameter and 45 cm high) was used (Morris, 1981). A transparent platform (10 cm in diameter and 25 cm high) was set inside the tank. The tank was filled to a height of 27 cm with water at approximately 23°C. The platform's surface was placed 2 cm below the water surface. The pool was located in a large room, in which there were many cues external to the maze. Positions of the cues were unchanged throughout the training.

For each training session, the animal was placed in the water so that it faced the wall of the pool. The platform was located in a constant position in the middle of one quadrant. In each training session, the latency to escape onto the hidden platform was recorded. If animal found the platform, it was allowed to remain there for 30 sec and then returned to its home cage. If animal was unable to find the platform within 300 sec, the training session was terminated and a maximum score of 300 sec was assigned.

Active avoidance test

The active avoidance test was performed according to the minor modification of the method reported by Nakamura *et al.* (1992). The apparatus consisted of a shuttle box (66 × 25 × 30 cm) with two identical compartments separated by a stainless steel plate. A house light attached to the ceiling of the shuttle box was used as the conditioned stimulus (CS), which was presented for 5 sec. If the rat crossed to the other compartment during the CS, an avoidance response was recorded. Otherwise, a foot-shock (0.7 mA, maximum 10 sec) was delivered through the grid floor as the unconditioned stimulus (UCS). Each rat was given 50 trials daily for 5 days with a fixed intertrial interval of 10 sec. To avoid the differences in each rat, rats were avoided if each of "avoidance" and "no response" responses in the individual rat is simultaneously more than 30% (15 trials at each behavior) in final training session. In the terminology, an "Avoid" transfer was used when the rat makes a transfer when the light starts. An "Escape" transfer was used when the rat transfers after the shock stimulus begins. However, if the rat remains in the original compartment and receives the 10 sec of shock, a "No response" was used.

Passive avoidance test

Two-compartmented box were used. One end of the box was black and dark inside; the other was well

illuminated. There was a guillotine door between the two compartments. Before the acquisition trial, each rat was accommodated in the box for 15 min. On day of the acquisition trial, each rat was placed in the dark chamber for adaptation. After a 120-sec adaptation period, the light was on and the guillotine door was open. Rat was allowed to enter the dark chamber and the initial latency time to enter the dark chamber was recorded as "naive time". Immediately after the rat entered the dark chamber, the guillotine door was closed and the electric foot shock (0.70 mA) was delivered to the floor grids for 5 sec. The animal was then put back into the home cage. Exactly 1.5 h and 24 h later for short- and long-term retention, retention latency time was measured in the same way as in the acquisition trial except delivering the foot shock, and the latency time was recorded to a maximum of 300 sec.

Statistical analysis

Data were expressed as mean ± S.E.M. Data from the water maze test and active avoidance test were analyzed by Student's t-test. Data from the passive avoidance test were recalculated into medians and interquartile ranges and were analyzed using Mann-Whitney U-test for the paired comparisons.

RESULTS

The latencies (to escape onto the hidden platform) in each training session of the water maze test are shown in Fig. 1. The latencies in the CSF-treated group on the first day of training were not different from those in the AF64A-treated group. During repeated training, the latencies in the AF64A-treated group were more slowly shortened than those in the CSF-treated group. From the 4th training session, impaired performance was observed in the AF64A-treated group. Changes in the latencies of the water maze after the infusion of AF64A into the pretrained rats are shown in Fig. 2. Seven days after the infusion, the latencies in the AF64A-treated group were significantly delayed. However, the latencies between two groups were not different at the next day session of the water maze.

Fig. 3 shows the mean escape latency times in the

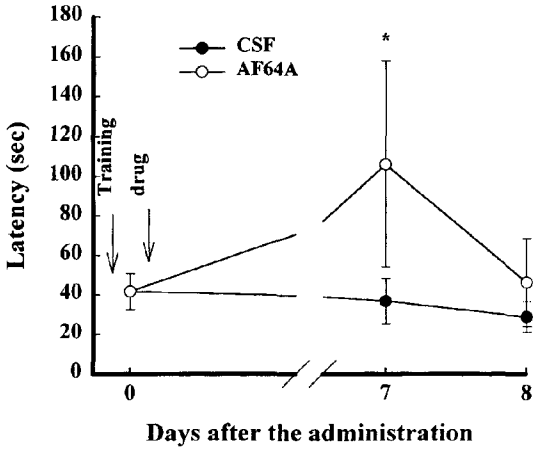

Fig. 2. Swimming behaviors after administration of AF64A in pretrained rats. Rats were pretrained for 3 days and then AF64A were infused into lateral ventricle. Seven days after the infusion, the water maze test in each rat was performed. Values are mean ± SEM. for 4 to 5 rats. The arrow indicated the infusion time with either artificial CSF or AF64A. *P< 0.05 compared with the artificial CSF-treated rats.

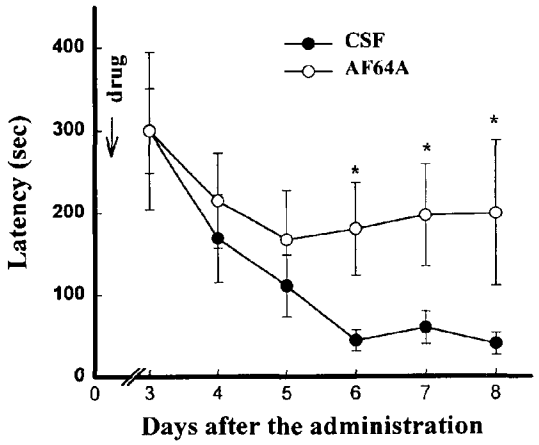

Fig. 1. Swimming behaviors after administration of AF64A in rats. Rats were treated with the infusion of AF64A into lateral ventricle. Three days after, rats were trained to find the platform for 6 days. The performed time was measured in each rat. Values are mean ± SEM. for 4 to 5 rats. The arrow indicated the infusion time with either artificial CSF or AF64A. *P<0.05 compared with the artificial CSF-treated rats.

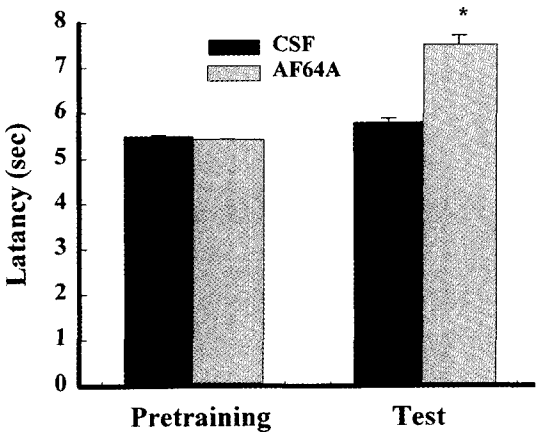

Fig. 3. Effects of AF64A administration on active avoidance escape latency time. Rats were pretrained for 5 days and then infused with AF64A. Seven days after the treatment, the escape latency was measured in each rat. Values are mean ± SEM. of 8 or 9 rats. *P<0.05 compared with the artificial CSF-treated rats.

active avoidance test after AF64A infusion. The escape latency times in AF64A-treated groups were significantly delayed to 140.8% compared to those in the pretraining or the CSF-treated groups. However, those in CSF-treated groups were not altered. After the responses between pretraining and testing in each rat are further analyzed, changes in the ratio of the sum of percentage of each behavior between pretraining and testing in each rat are shown in Fig. 4A. The "no response" in the testing period of the CSF-treated group was decreased, but that in the AF64A-treated group was not altered. The escape behaviors in both groups were unchanged compared to the pretraining period. The avoidance responses in both groups were increased in the testing period. However, the increasing rate of the AF64A-treated rats was lower than that of the control group. For the analysis of each trial, the total trials (50 trials) are divided into two sections (25 trials each). Fig. 4B shows changes in each behavior of the first half trials. The "no response" in the testing period of the CSF-treated group was decreased. However, that in the testing period of the AF64A-treated group was remarkably increased. The escape responses were not changed in both groups. Although the avoidance responses in both groups were increased, the increasing rate of the AF64A-treated group was lower than that of the control group. Fig. 4C shows changes in each behavior of the last half trials. Both of the "no response" and the escape responses in the testing period of CSF- and AF64A-treated groups were similarly decreased compared to the pretraining period. However, the avoidance responses of the last half trials in both groups were similar to the those of first half trials.

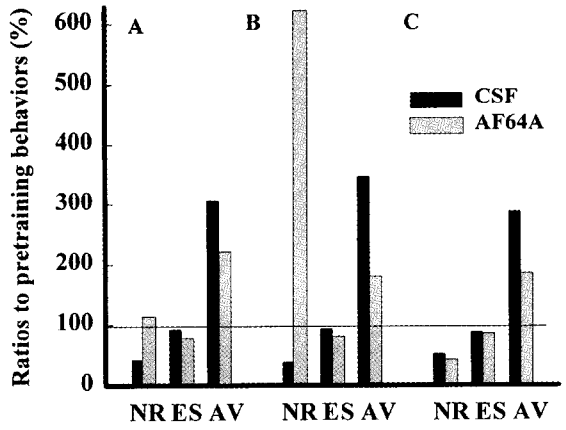

Fig. 4. Effects of i.c.v. administration of AF64A on the total (A), the first (B) and the last half (C) trials of active avoidance test. Legend is the same as Fig. 3. The percentages of each action mode were measured in each rat. The changes in percentage of each action mode of testing periods are obtained from the basis on the sum of the frequency in the pretraining periods. NR, ES and AV indicate "No response", "Escape" and "Avoidance", respectively.

The latencies in each training session of the passive avoidance test are shown in Fig. 5. The latencies in the AF64A-treated group at 1.5 h (for the short term learning) after the electric shock were significantly shortened compared to the CSF-treated group. However, the latencies in the AF64A- and the CSF-treated group at 24 h (for the long term learning) after the electric shock were comparable each other. Changes in the latencies of the passive avoidance after the infusion of AF64A into the pretrained rats are shown in Fig. 6. Seven days after the infusion, the latencies in the AF64A-treated group were significantly shortened.

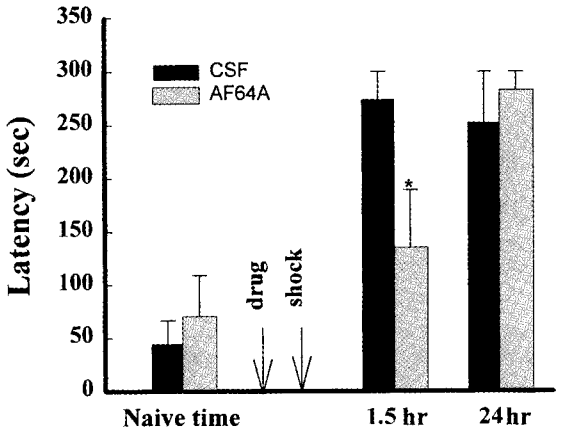

Fig. 5. Passive avoidance behaviors after administration of AF64A in rats. Rats were given the electronic shock at seven days after the AF64A infusion. Either 1.5 h or 24 h after the shock, the same passive avoidance test was performed in each rat. The performed time was measured in each rat. Values are mean ± SEM for 6 rats. *P<0.05 compared with the artificial CSF-treated rats.

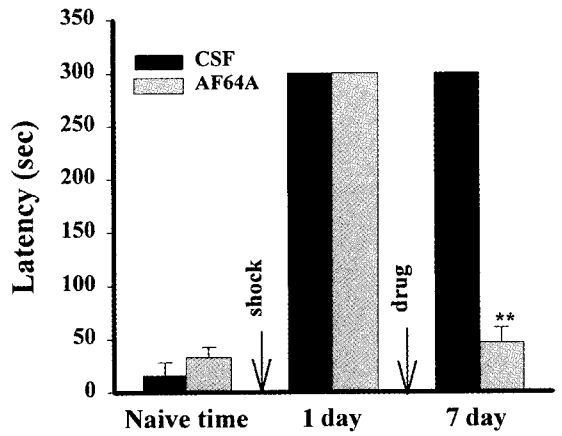

Fig. 6. Passive avoidance behaviors after administration of AF64A in rats recognizing the shock. AF64A or artificial CSF was infused in rats recognizing the electronic shock. Seven days after the infusion, the same passive avoidance test was performed in each rat. The performed time was measured in each rat. Values are mean ± SEM for 5 or 6 rats. **P<0.01 compared with the artificial CSF-treated rats.

DISCUSSION

Acquisition of behaviors is usually thought of largely instinctive. A cholinergic neurotoxin, AF64A, has been reported to induce the destruction of the presynaptic terminals in cholinergic nervous system and the decrease in various cholinergic indices (Fisher *et al.*, 1982; Ransmayr *et al.*, 1992). Although the various neuronal systems were changed by the administration of AF64A (Gaal *et al.*, 1986; Lim *et al.*, 1996; Ma and Lim, 1997; Potter *et al.*, 1989), the function of the septohippocampal cholinergic system has been also altered (Chrobak *et al.*, 1988, Nakahara *et al.*, 1988). It is well known that the septohippocampal cholinergic system is a critical neuronal substrate of memory processes. In the acquisition trials after AF64 treatment, latency to escape in water maze test is delayed from the fourth performance and that in passive avoidance test is shortened at an early hour after the shock experience. Thus, the present results imply that intracerebroventricular injection of AF64A impairs acquisition. It has been reported that dementia due to Azheimer's disease was less common in well-educated people than in uneducated people (Zhang *et al.*, 1990). From that point of view, it is suggested that the establishment of acquired neural circuits requires the participation of more neuron than the activation of well-established acquired circuits and also the destruction of neurons usually affects acquisition more than retention. One fact of amnestic syndrome is that memory established in the distant past is often well retained while new learning is difficult (Vanderwolf and Cain, 1994). Therefore, the results suggest that AF64A might be a useful agent for the amnestic animal model. Also the acquisition in water maze test was continuously impaired and that in a passive avoidance test was regained at the later hour after the shock experience. These results suggest that AF64A may impair the more severe deficit in spatial learning than in sensory learning. However, the acquisition of T-maze test and a passive avoidance test were reported to be unaffected by the AF64A treatment (Chrobak *et al.*, 1988; Nakahara *et al.*, 1988). Although the exact reasons for the discrepancy are not known, the discrepancy might be due to the applied maze tests (water maze and T maze) and the time schedule performed in a passive avoidance test (1.5 h and 24 h after the treatment).

Following a memorable experience, a permanent memory trace might usually be constructed. Although the impairment in the retention of memory by the drug treatment is not entirely due to the elimination of stored information from the brain (DeVietti and Kirkpatrick, 1976; Vanderwolf and Cain, 1994), the present results reveal that the latencies to escape in water maze and active avoidance tests were delayed and that the latency to escape in passive avoidance test was shortened after the injection of AF64A into the pretrained rats. Thus, the present results imply that AF64A also impairs the retention of memory. It has been reported that the retentions of memory by AF64A administration were impaired in T-maze, avoidance tests and Morris water maze test (Chrobak *et al.*, 1988; Lermontova *et al.*, 1998; Lim *et al.*, 1995; Mannisto *et al.*, 1994). Thus, the present results are agreed with those reports. However, the present results indicate that the active avoidances in both groups are increased. It has been reported that the response at active avoidance test was even increased, but not the total responses (Nakahara *et al.*, 1988). Although the results are agreed, the increasing active avoidance response in AF64A-treated group is lowered than that in the control. The discrepancy may due to the analyzing methods. Furthermore, the present results reveal that the latencies in the second performance at water maze and passive avoidance tests were comparable with the control groups. Furthermore, during continuous trials at the active avoidance test, the initial increase of the "no response" responses in the first half trials is disappeared in the last half trials. These imply that the impaired memory is restored during testing. Therefore, the present results suggest that although the acquired memory is impaired by AF64A, but the new learning is quickly adapted after the first retrain.

Altogether, AF64A may impair the learning and the memory related to space and unpleasant stimuli. Although AF64A-induced deficits in memory are not permanent but transitory, AF64A might be a useful agent for the animal model of learning and memory.

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