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Effects of Ethylcholine Aziridinium, Scopolamine and Morphine on Learning Behaviors in Morris Water Maze.

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To assess the learning behaviors of the various chemicals, such as ethylcholine aziridinium (AF64A), scopolamine and morphine, the chemicals were administered into either rat or mice. And water maze tests were performed before and during drug administration. In AF64A-treated groups (3 nmol/each ventricle), the latencies to escape was significantly increased in both of the pretraining- and posttraining groups. In scopolamine-treatment (2 mg/kg, sc) to the pretrained group, the latency to escape was significantly shortened after the acute administration of scopolamine. However in subacute treatment group with scopolamine, the latency to escape was significantly increased. In morphine-treated groups (10 mg/kg, ip), the latency to escape was significantly increased after the acute administration. However in subacute treatmment with morphine, the latency to escape was not changed. The results indicate that each chemical induces the learning impairment. However the chemical-induced learning impairment may have different characteristics upon the exposed chemical. Also the results suggest that both the motivation and the retrieval of memory might be impaired by AF64A.

Key words : Ethylcholine aziridinium, Scopolamine, Morphine, Morris water maze

INTRODUCTION

In alzheimer disease (AD), the disturbances of the picture are dominate in the early symptoms. But the main symptom is intelligence impairment, especially memory disturbances. Although the causes of the disease are still controversial, it is known that the central cholinergic nervous system are closely related to the learning impairment. The various changes in cholinergic nervous systems, such as the loss of nerve terminals, the reduction of muscarinic receptors, and the loss of acetylcholinesterase (AChE) and choline acetyl transferase (CAT) activities, in forebrain and hippocampus were reported in either AD-related biopsy brain tissue or animal models (Bartus *et al.*, 1982; Coyle *et al.*, 1984; Mash *et al.*, 1985; Sims *et al.*, 1983).

It has been reported that several chemical substances were suggested to induce the amnesia in the animals. Ethylcholine aziridinium (AF64A), scopolamine, and morphine have induced the learning impairment and their actions were the reduction of the central cholinergic activities with the specific cholinotoxin (Chrobat *et al.*, 1988; Nakahara *et al.*, 1988), muscarinic antagonist (Spencer and Lal, 1983; Spencer *et al.*, 1985), and the indirect reduction of acetylcholine release (Crossland and Ahmed, 1984; Izquierdo, 1979), respectively. Also the chemically induced learning impairments have been demonstrated using several measurements, such as passive avoidance paradigm, radial arm maze test, and Morris water maze test (Bailey *et al.*, 1986; Brandeis *et al.*, 1986; Flood and Cherkin, 1986; Nishimura *et al.*, 1990). However it has been reported that the degrees of learning impairments were differed upon the measured methods of behaviors, dose and injected route of substances (Buresova *et al.*, 1986; Nishimura *et al.*, 1990). To improve the usefulness of these amnestic animal models, it is necessary to measure the difference using the same measurement.

Therefore this study were designed to assess the effects of AF64A, scopolamine and morphine on the learning impairments using Morris water maze test.

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MATERIALS AND METHODS

Animals and materials.

Four male Sprague-Dawley rats weighing 200-250 g and ten male mice weighing 20-25 g were housed per cage at 22±2°C on a 12 h light/12 h dark schedule (8:00 a.m.-8:00 p.m.). Animals were freely accessible on food and water.

Acetylethylcholine mustard-HCl were purchased

from Research Biochemical Inc. (Wayland, MA). Ethylcholine aziridinium (AF64A) is synthesized according to the method of Mantione *et al.* (1983). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Animal treatment.

For the administration of AF64A, all surgical procedures were performed on male Sprague-Dawley rats with initial weights 250-300 g. Rats were anesthetized with Equithsin and mounted in a David Kopf stereotaxic apparatus. The skull was exposed and a guide cannule was implanted according to Paxino and Watson (1986) through the dural surface into the lateral ventricle with respect to bregma at the following coordinates: 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. Penicilline 30,000 I.U. was administered to protect from infection after surgery. Before the injection of AF64A, the rats were allowed to recover from surgery for 4 days, housed singly in their cages. AF64A was infused in both ventricle with the rate of 0.5 μl/min (3 nmol/each side) at four days after the surgery. The control groups were infused with the artificial cerebrospinal fluid. At four to seven days after the infusion of AF64A, water maze behavioral tests were performed. For the retrieval tests, rats were pretrained before the surgery and the processes were same as the above method described.

For the administration of scopolamine, rats were pretrained in the water maze apparatus. Four days after the training, 2 mg/kg of scopolamine was subcutaneously administered at thirty mins before the water maze test. In subacute treatment group, water maze training was performed every day at 30 min after the administration of scopolamine. The control rats were administered with saline.

For the administration of morphine, mice were treated with 20 mg/kg of morphine, i.p., at 30 min before the water maze test. In subacute treatment group, water maze training was performed every day at 30 min after the administration of morphine. The control rats were administered with saline.

Water maze task

A circular water tank (140 cm in diameter and 45 cm high) was used for rats (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 surface of the water. Also a rectangular water tank (60 cm in length, 30 cm in width and 36 cm in high) was used for mice (Yamada *et al.*, 1992). The water tank was divided into 4 spaces. The interspaces were divided by the plate (18 cm in length, 23.2 cm in high and 1 mm in thickness). Each plate was located in the opposite site of the tank. A transparent platform (8.5 cm in length, 5 cm in width and 1 cm in thickness) was set inside the tank and the platform surface was placed 1 cm below the surface of the water. The pool was located in a large room, in which there were many cues external to the maze, which were visible from within the pool and could be used by the rat or the mouse for spatial orientation. 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 for rat and the corner of the other site for mouse. 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 for rat and 180 sec for mouse, the training session was terminated and a maximum score of 300 sec for rat and 180 sec for mouse was assigned.

Statistical analysis

Data were expressed as medians and interquartile ranges. All data were analyzed by using Mann-Whitney U-test for the paired comparisons. $P<0.05$ was used as the criterion for statistical significance.

RESULTS

As shown in Fig. 1, the latencies to escape after the infusion of AF64A into lateral ventricle was increased. When water maze test was performed once a day from three days after AF64 infusion, the latencies to escape in the control and the treated group were 43.5±13.5 sec and 183.5±53.6 sec, respectively, at 5th days (Fig 1A). The latencies to escape in the treated group was significantly increased. Even in the pretrained groups, the latencies to escape in the AF64A-treated rats were also significantly increased. The latencies to escape were 27.0±5.6 sec and 106.5±52.1 sec from the control and the treated groups, respectively, 6 days after the AF64A-treatment (Fig. 1B).

As shown in Fig. 2, the latencies to escape were differently affected by the numbers of the administration with scopolamine. When scopolamine was acutely administered 30 min before water maze task, the latencies to escape was significantly shortened. The latencies to escape in the control and the scopolamine-

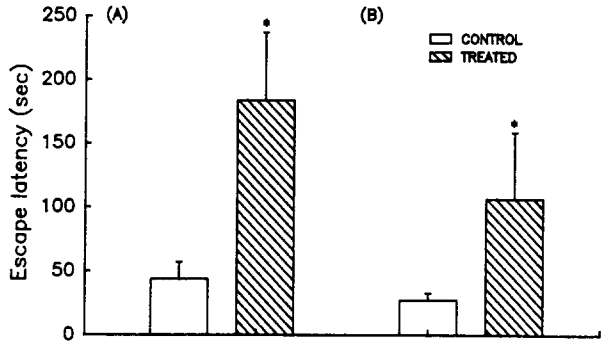

Fig. 1. Effects of AF64A administration on water maze behaviors in the naive (A) and the pretrained (B) rats. (A) Rats were treated with AF64A. Three days after, water maze tests were performed for 5 days. (B) Rats were pretrained for 3 days and then infused with AF64A (3 nmol/each ventricle). Six days after the infusion, water maze tests were performed. The performed times (latencies) were measured at each rat. Values are mean S.E.M. for six to seven rats. *P <0.05 compared with corresponding control values

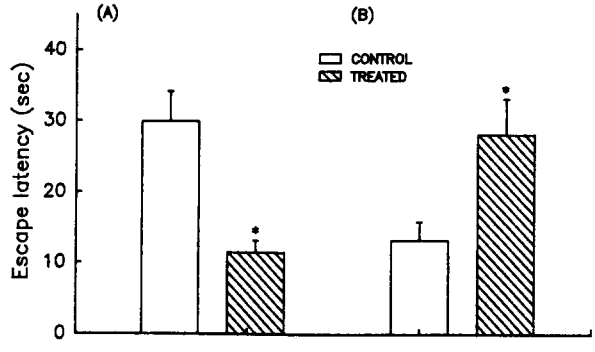

Fig. 2. Effects of acute (A) and subacute (B) administration of scopolamine on water maze behaviors in the pretrained rats. Rats were pretrained for 3 days and then administered with scopolamine, 2 mg/kg, sc, for acute (A) and 4 days (B). Thirty min after, water maze tests were performed every day. The latency to excape was measured at each rat. Values are mean S.E.M. for six to seven rats. *P<0.05 compared with corresponding control values

treated rats were 29.9±4.2 and 11.4±5.1 sec, respectively (Fig, 2A). However when rats were treated for daily 4 days with scopolamine, the latencies to escape in the treated group was significantly increased. Those in the control and the subacutely scopolamine-treated rats were 13.2±2.6 and 28.1±5.1 sec, respectively (Fig. 2B).

As shown in Fig. 3, the latencies to escape were changed by the treatment with morphine. When morphine was acutely administered 30 min before water maze task, the latencies to escape was significantly increased. The latencies to escape in the control and the morphine-treated mice were 60.9±19.3 and 137.6±25.5 sec, respectively (Fig, 3A). However when mice was treated for daily 5 days with morphine, the latencies to escape in the treated group was not

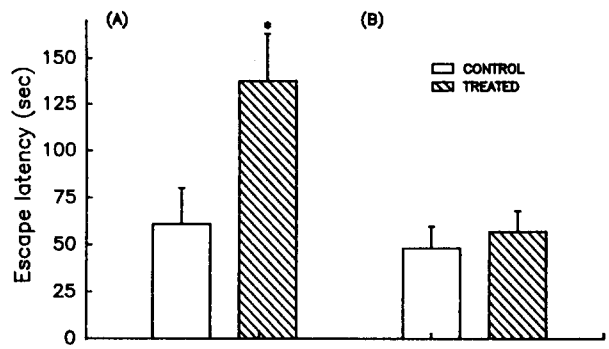

Fig. 3. Effects of acute (A) and subacute (B) administration of morphine on water maze behaviors in mice. Mice were administered with morphine, 20 mg/kg, ip, for acute (A) and 4 days (B). Thirty mins after, water maze tests were performed every day. The latency to excape was measured at each mouse. Values are mean S.E.M. for nine to ten mice. * P<0.05 compared with corresponding control values

changed (Fig.3B).

DISCUSSION

The present results demonstrate that various chemical agents induce the changes in the learning and the retention of memory in water maze test. However the altered effects were depended upon the administered agents, such as agent itself and the numbers of administration.

It has been reported that AF64A has induced the decreases in choline uptake and the destruction of the presynaptic terminals in cholinergic nervous systems (Bartus *et al.*, 1982; Leventer *et al.*, 1987; Ransmayr *et al.*, 1992). Also the loss of memory in AF64A-administered rats has been reported to be induced in T-maze and passive avoidance tests (Chrobak *et al.*, 1988; Nakahara *et al.*, 1988). The present results is agreed with those reports. Furthermore it suggests that the learning to escape might be decreased in AF64A-treated rats. In our preliminary results, the choline uptake and acetylcholinesterase activities in hippocamus shows the significantly decrease in AF64A-treated rats (data not shown). However it has been reported that the administration of AF64A into lateral ventricle induces the enlargement of ventricle and suggested that AF64A has the direct as well as the indirect effects in the nervous systems (Chrobak *et al.*, 1989). Although it need to investigate the indirect effect of AF64A, the decreased learning abiltities after the administration of AF64A might be due to the decrease in the central cholinergic nervous activities.

It has been reported that scopolamine induced the impairments of retrograde memory (Loullis *et al.*, 1983). However Flood and Cherkin (1986) reported that the memory impairments were induced in the high dose of scopolamine, while the memory retention was facilitated at the low dose of sco-

polamine. The present result indicate that the acute and subacute administration with scopolamine differently affect the memory retention. It has been reported that the release of neurotransmitters were regulated by presynaptic receptors and the release of neurotransmitters was increased by antagonists (Starke, 1981). Also the upregulation of receptors was reported by the repetitative administration of antagonists (Raiteri *et al.*, 1981). Although it is needed to further study the exact biochemical changes after the subacute administraion, the present result implys that the numbers of scopolamine administration might induce the different response in memory retentions at the present dose.

It has been reported that the motivations in the aged rats were more impaired than those in the young rats and the changes in opioid systems were related to the age (Jensen *et al.*, 1980). Furthermore the impairment of memory was reported by the administration of morphine (Gallagher and Kapp, 1978; Kaneto, 1990). However it has been reported that the high dose of morphine after the pretraining induced to facilitate the memory (Mondadori and Waser, 1979). Also it has been reported that the administration of morphine before the pretraining delayed the motivation, while the administeration of morphine after the pretraining did not affect the memory response in mice (Nishimura *et al.*, 1990). The present results are agreed with the latter report; the motivations are delayed in the acute treatment. Since the water maze was performed at every day during subacute administration, no difference in the latency to excape in the subacute administration group implys that either memory ability or retentions are not affected by subacute administration of morphine. Crossland and Ahmed (1984) reported that morphine acts on central cholinergic nervous system and induces the decreased release of acetylcholine. Although the exact mechanisms are unknown, the decreased activities in cholinergic nervous system by the acute administration of morphine might be induced the reduction of the motivation.

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