Effects of Physostigmine on AF64A-Induced Impairment of Learning Acquisition in Rats

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Abstract—Ethylcholine aziridinium ion (AF64A), a putative cholinotoxin, was administered into the cerebroventricles of rats, and the effects on learning behaviors were observed. AF64A caused the impairment of learning acquisition in both passive and active avoidance responses. Physostigmine, a cholinesterase inhibitor, antagonized these changes at the doses of 0.03 to 0.1 mg/kg. Our behavioral study may indicate that the central cholinergic system might play a role in AF64A-induced impairment.

Ethylcholine aziridinium ion (AF64A) is an analog of choline and reported as a cholinergic neurotoxin (1, 2). It has been known that AF64A inhibits high-affinity choline transport (2, 3). The compound also possesses inhibitory action toward the synthesizing enzyme for acetylcholine (ACh), choline acetyltransferase (ChAT) (4), although it does not influence [3H]QNB binding, a postsynaptic marker of the ACh system (5). Furthermore, when a suitable amount of AF64A was injected into some regions of the brain or cerebroventricles, it caused a long-term hypofunction of the cholinergic system without any effects on other neurotransmitter systems, e.g., the norepinephrine, dopamine, serotonin and so on (6-8).

While biochemical studies have been revealed many of the characteristics of AF64A, little is known about its behavioral properties. Furthermore, the effects of drugs on AF64A-induced behavioral changes are unknown. So in this paper, we report that AF64A exerted an influence on learning in two kinds of avoidance responses, and these changes were reversed by a cholinesterase inhibitor, physostigmine, in rats.

AF64A was prepared according to Fisher et al. (5) from acetyl-AF64, which was synthesized in our laboratories and identified by nuclear magnetic resonance. The amount of AF64A was titrated by the method of Hirst and Jackson (9). Male Fischer 344 rats (9-12 weeks old, 200-400 g; Charles River) were anesthetized with pentobarbital Na (50 mg/kg, i.p.) and placed in a stereotaxic apparatus. AF64A was injected bilaterally into the cerebroventricles (AP: -0.8 mm, L: 2.0 mm from bregma, V: 3.0 mm from dura; Pellegrino et al. (10)) over a period of 60 sec. One ul was injected in each side.

We used the minimal dose of AF64A to cause a stable impairment in each avoidance test, and these tests were performed when the central cholinergic system was thought to be fully damaged by AF64A as reported elsewhere (11).

One week after treatment with 8 nmoles AF64A or saline, the passive avoidance test was carried out. A step-through type apparatus was used that consists of light and dark compartments. Each animal was placed in the light side of the chamber, and a guillotine door was opened. When a rat entered into the dark side, the door was shut, and a foot shock (1.0 mA, 5 sec) was delivered. Twenty-four hours later, a subject was placed into the light side again, and the length of time before he entered the other side was recorded.

Rats injected with 2 nmoles of AF64A or saline were subjected to the active avoidance test 2 weeks later. One-way shuttle boxes (Lafayette) were used. They were con-
trolled automatically by a Cromemco Z-2 microcomputer. The experimental schedule consisted of the following: the conditioned stimulus was a 5 sec light followed by the unconditioned stimulus, a foot shock (AC 90 V, 5 sec). During the presentation of both stimuli, a shelf was made available by a sliding wall. When an animal got on the shelf, a microswitch reacted and a response time was automatically recorded. The response during the conditioned stimulus was considered the conditioned avoidance response (CAR). The inter-trial interval was 50 sec. Trials were repeated 30 times consecutively.

Rats treated with AF64A showed hypersensitivity, which developed into convulsions in severe cases. These signs became gradually weaker.

In the training trial of the passive avoidance test, AF64A-treated rats took a longer time to enter the dark compartment (mean±S.E.: 66±8.7 sec) than saline-treated rats (34±6.0 sec). In the retention trial, the saline-treated control group showed a long response latency (512±37.4 sec, median: 600) (Fig. 1), which means that these animals learned the task sufficiently. In contrast, the AF64A group showed a significant shortening of response latency (212±46.5 sec, median: 137) compared with the control group.

Physostigmine was administered intraperitoneally 30 min before the training trial. Physostigmine showed a significant reverse effect against AF64A-induced impairment at the dose level of 0.1 mg/kg (455±65.0 sec, median: 600) in the retention trial, while it had no effect on AF64A-induced prolongation of response latency in the training trial. However, at the higher or lower doses used, we could not find any reverse effect of physostigmine.

In the active avoidance test, saline-treated control animals acquired 63±2.2% of correct CAR in 30 trials (Fig. 2). On the other hand, the AF64A-treated group showed a significant decrease of CAR acquisition (41±3.4%). However, there was no difference between the AF64A group and the control group in the mean number of trials without responses. This change induced by AF64A was significantly antagonized by physostigmine injected 30 min before the test at the dose of 0.1 mg/kg (54±4.5%).

We also made an analysis of data, calculating the number of trials required for the subject to perform two consecutive CARs. Rats in the saline-treated group showed 80% CAR after two consecutive CARs. We thought that two consecutive CARs must serve as the criteria for the establishment of learning. The AF64A-treated group indicated a significant increase before they achieved two consecutive CARs (18.1±1.07 trials) compared with the control group (11.2±0.65 trials). In the measurement of this data, physostigmine also showed a significant reverse effect against the increase of trials induced by AF64A at the dose levels of 0.03 (14.0±1.80 trials) to 0.1 mg/kg (13.5±1.67 trials).

When AF64A is injected intraventricularly, the damage to the hippocampus is greater than that to any other part of the brain.
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Fig. 2. Effects of physostigmine on AF64A-induced acquisition impairment of active avoidance response in rats. Each column represents the mean value with S.E. Left: The total CAR % in 30 trials is represented. Right: The number of trials to criteria is represented. The details of the criteria are described in the text. *: Significantly different from the value in the AF64A (i.c.v.) and saline (i.p.)-treated group at P<0.05.

Vickroy et al. (11) has reported that intraventricular AF64A caused a selective deficit to the hippocampal cholinergic system in rats. In our preliminary study, the injection of 2 or 8 nmoles of AF64A showed a 25 or 50 percent decrease of ChAT activity in the hippocampus, respectively, without any changes of ChAT activities in the cerebral cortex and striatum 4 weeks after intraventricular injection. Therefore, the hippocampal cholinergic system may play a role in the behavioral changes in the present study.

The effects of intraventricular AF64A on the passive avoidance test have been reported by some investigators. Walsh et al. (8) reported that 15 or 30 nmoles of AF64A induced the impairment of the passive avoidance task in rats, although they used higher doses than that used in the present study. Pope et al. (12) also reported the AF64A-induced impairment of the passive avoidance test in mice.

We could not completely exclude the possible contribution of hyperactivity to acquisition impairment in this task. Hyperactivity was shown in the rats treated with 8 nmoles of AF64A. However, physostigmine did not inhibit hyperactivity at the dose level that was effective for improving AF64A-induced impairment (our preliminary data). So the improving effect of physostigmine can not be explained solely by its effect on locomotor activity.

As far as we know, there is still no data about the effects of intraventricular AF64A on the active avoidance response. In this study, we found that AF64A caused an acquisition deficit in the active avoidance response. Furthermore, AF64A did not have any influence on the number of trials without response in the acquisition process of the active avoidance task. This result suggests that the deficit in this task is not due to the inability to perform the avoidance response or to changes in sensitivity to the foot shock.

Some reports on lesion studies, which would disagree with our results, indicated that a hippocampal deficit facilitated learning in the active avoidance task (13, 14). The discrepancy may be due to the method used: they used a two-way shuttle box, which is different from the one-way shuttle box used in our method. On the other hand, Bailey et al. (15) studied the effects of AF64A injected in the dorsal hippocampus in the active avoidance task in rats. They used the two-way shuttle box with a guillotine door in the center, which was opened only during the presentation of stimuli. They found that
AF64A produced an acquisition deficit. This corresponds with our result. In our method, animals could not react (climb on the shelf) except during the presentation of the conditioned or unconditioned stimuli.

Physostigmine at 0.03 to 0.1 mg/kg had improving effects on the impairments induced by AF64A in both the passive and active avoidance tasks. These dose levels of physostigmine did not produce a remarkable change in general behavior. However, higher dose of physostigmine showed no improving effects and its dose-response curves were inverted U-shaped ones. When physostigmine was injected to rats at the dose of more than 0.3 mg/kg, various peripheral signs such as miosis, salivation, lacrimation or fasciculation developed. These signs were disadvantageous to the improving effects of physostigmine.

The animal models treated with AF64A continue to be studied as a promising disease model for central cholinergic hypofunction, especially Alzheimer's disease (16). However, there is no report demonstrating drug effects on this model to date. Our results, which showed that AF64A-induced changes were antagonized by physostigmine, encouraged us to use this animal model in evaluating new drugs. Further studies about the effects of other drugs on this model are necessary.

References
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