Amelioration of Experimental Amnesia (Passive Avoidance Failure) in Rodents by the Selective M₁ Agonist AF102B

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Abstract—Effect of AF102B (cis-2-methylspiro-(1,3-oxathiolane-5,3')-quinuclidine) on experimental amnesia was examined using a passive avoidance task in rodents. The amnesia was produced by anti-cholinergic agents, AF64A (intracerebroventricularly) and scopolamine (subcutaneously). AF102B ameliorated the memory deficits in AF64A-treated rats at 0.1–1 mg/kg, i.p. and at 1–5 mg/kg p.o. and in scopolamine-treated mice at 1–10 mg/kg, i.p. These results suggest that AF102B may compensate for central cholinergic defects and could be developed as a possible therapeutic drug for senile dementia of the Alzheimer type.

The most notable symptoms in senile dementia of the Alzheimer type (SDAT) are generally characterized by a memory loss of recent events and cognitive deficits. In SDAT, abnormalities of several neurotransmitter systems in the brain have been reported, including cholinergic, noradrenergic, serotonergic and somatostatinergic systems (1, 2). Among them, the abnormality in cholinergic system is generally greater and more consistent and is found to correlate well with the cognitive deficits (1–3). Moreover, ample evidence has suggested that cholinergic function is involved in memory and cognitive functions in humans as well as animals (4). These observations rationalize clinical trials with compounds that enhance cholinergic transmission such as acetylcholinesterase inhibitors (physostigmine, tetrahydroaminoacridine) and muscarinic agonists (arecoline, oxotremorine, RS 86). A substantial number of studies have reported modest but significant improvements, but not without suffering from serious limitations such as short duration of action and a narrow therapeutic window (1, 5). These results encouraged us to develop a cholinomimetic compound as a therapeutic drug for SDAT. Since no conclusive animal models are yet available, the efficacy of compounds should be evaluated in various ani-

mal models before final clinical trials. Scopolamine-induced experimental amnesia mice (SCP-mice) are widely used as an animal model for memory disorders including SDAT (4). Intracerebroventricular (i.c.v.) injection of a putative cholinotoxin AF64A brings about a cholinergic hypofunction in the hippocampus together with memory deficits, indicating that AF64A-treated rats (AF64A-rats) are workable animal model for SDAT (6). In the present paper, we have examined effects of AF102B, a novel muscarinic agonist of the M₁-type receptor, on passive avoidance failures in AF64A-rats and SCP-mice. A preliminary report of part of this work has been published elsewhere (7).

AF102B (cis-2-methylspiro-(1,3-oxathiolane-5,3')-quinuclidine hydrochloride hemihydrate) and acetyl-AF64 were synthesized and provided by Dr. A. Fisher (Israel Inst. Biol. Res., Ness-Ziona, Israel). (–)-Scopolamine hydrochloride, oxotremorine sesquifumarate and arecoline hydrobromide were purchased from Sigma (St. Louis, U.S.A.). AF64A (ethylcholine aziridinium ion) was freshly prepared by alkaline hydrolysis of acetyl-AF64 (8). The surgery and passive avoidance test were carried out as described elsewhere (8). Briefly, male Sprague-Dawley rats (200–250 g, Charles

River Japan, Atsugi) were bilaterally injected with AF64A (3 nmol/2 μ l per side) or saline solution (vehicle) into the lateral ventricles. Rats were subjected to a one-trial step-through passive avoidance task 19–21 days after surgery. AF102B (0.1 or 1 mg/kg), oxotremorine (0.03 or 0.1 mg/kg), arecoline (1 or 5 mg/kg) or saline solution (vehicle) were intraperitoneally administered immediately after the acquisition trial. In some experiments, AF102B (0.2, 1 or 5 mg/kg) or distilled water (vehicle) were perorally administered 30 min before the acquisition trial. The retention trial was carried out 24 hr after the acquisition trial. Latency time was recorded up to a maximum of 600 sec.

Male ddy mice (25–30 g, Shizuoka Laboratory Animal Center, Hamamatsu) were subjected to a one-trial passive avoidance task. A step-through type apparatus for mice was used. Scopolamine (1 mg/kg) was subcutaneously injected 30 min before the acquisition trial. For control mice, saline solution was injected. After a 30-sec habituation in the light chamber, each mouse was

allowed to enter the dark chamber by removing a guillotine door. Upon entry into the dark chamber, the mouse received foot shock (250 V, 50 HzAC) for 1 sec. Initial latency time to enter the dark chamber was recorded. The mice that had an initial latency time of more than 60 sec were excluded from further experiments. AF102B (1, 5 or 10 mg/kg) or saline solution (vehicle) were intraperitoneally administered immediately after the acquisition trial. Retention of the passive avoidance task was evaluated 24 hr after the acquisition trial. Retention latency time was recorded up to a maximum of 600 sec.

In the retention trial, saline-treated control rats exhibited a long response latency of 600 sec (Fig. 1a). In contrast, the latency of AF64A-rats receiving vehicle was significantly shorter than that of control rats, in agreement with our previous result (8). AF102B (0.1 or 1 mg/kg, i.p.) administration to AF64A-rats produced a significant improvement of a passive avoidance response (Fig. 1a). Oxotremorine significantly enhanced the retention test performance of

Fig. 1. Effects of AF102B, oxotremorine and arecoline on retention latencies in AF64A-treated rats. Compounds were administered intraperitoneally (a) or perorally (b). Values represent the median with the number of animals in columns. Significantly different from AF64A-rats receiving vehicle: * $P < 0.05$, ** $P < 0.01$ (Mann-Whitney U -test).

AF64A-rats at 0.03 and 0.1 mg/kg, i.p. Arecoline also significantly reversed the retention deficits of AF64A-rats at 5 mg/kg, i.p., but not at 1 mg/kg, i.p. With respect to the initial latency in the acquisition trial, there was no significant difference among the three groups in each drug treatment.

When AF102B was perorally administered to AF64A-rats, it produced a dose-dependent amelioration of passive avoidance learning and memory deficits (Fig. 1b). AF102B significantly improved the avoidance performance of AF64A-rats at 1 and 5 mg/kg, p.o. When AF102B was administered at 0.2 mg/kg, p.o., slight but not significant improvement was found. In the acquisition trial, there was no significant difference in the latency time among the five groups, showing that preadministration of AF102B had no influence on the acquisition trial.

The retention latency of SCP-mice receiving distilled water (vehicle) was significantly shorter than of saline-treated control mice. AF102B (1, 5 or 10 mg/kg, i.p.) significantly enhance the retention test performance of SCP-mice (Fig. 2).

Fig. 2. Effects of AF102B on retention latencies in scopolamine-treated mice. Values represent the median with the number of animals in columns. Significantly different from SCP-mice receiving vehicle: * $P < 0.05$, ** $P < 0.01$ (Mann-Whitney U -test).

AF64A, an irreversible inhibitor of the high affinity uptake of choline, is reported to produce a selective reduction of presynaptic markers in the hippocampal cholinergic system in rats after i.c.v. injection (for review, see ref. 6). Leventer et al. (9) indicate that at low doses (≤ 5 nmol), the effects of AF64A appear to be limited to the cholinergic system, and that at higher doses, noncholinergic effects become prominent, although selectivity of AF64A towards the cholinergic system is not without controversy. We have recently observed that AF64A injected into the lateral ventricles (3 nmol, each side) impaired the cognition of rats in a passive avoidance task and in a delayed alternation task in a T-maze with a concomitant hypofunction in the hippocampal cholinergic system (8). Passive avoidance learning and memory deficits are ameliorated by physostigmine (8, 10). In the present study, muscarinic agonists improved the defective retention of AF64A-rats in the passive avoidance task. These results suggest that the cognitive impairment produced by AF64A may be due to AF64A-induced changes in hippocampal cholinergic activity rather than being caused by non-specific neural damage.

With respect to the doses of muscarinic agonists for alleviation of AF64A-induced passive avoidance failure, 0.1 mg/kg, i.p. (410 nmol) of AF102B and 0.03 mg/kg, i.p. (79 nmol) of oxotremorine had positive effects, but 1 mg/kg, i.p. (4200 nmol) of arecoline had negative effects. This seems partly due to a difference in their affinities to the muscarinic receptor. In our earlier studies on the inhibition of [3 H]QNB binding to receptors on rat cerebral cortex membranes and to the purified muscarinic receptor from porcine cerebrium, we found that the apparent K_i values in the membrane preparations and the purified receptor are 5.1 μ M and 10 μ M for AF102B, 1.3 μ M and 3.8 μ M for oxotremorine, and 15 μ M and 37 μ M for arecoline, respectively (11). When AF102B was intraperitoneally administered to AF64A-rats at 0.1–1 mg/kg or SCP-mice at 1–5 mg/kg, significant but not dose-dependent improvement was found in the passive avoidance task. It may be suggested that i.p. administration of AF102B is highly potent for amelioration of

the cognitive impairments caused by AF64A or scopolamine and that the efficacy of each dose has already reached plateaus in these experiments.

Scopolamine, a reversible inhibitor of muscarinic receptors, is known to disrupt cognitive function in humans well as in animals, presumably through central cholinergic dysfunction (1, 4). It is reported that a scopolamine-induced cognitive impairment is recovered by physostigmine (4). AF102B also improved the scopolamine-induced amnesia of mice in a passive avoidance task. Taken together, our present results have provided further evidence suggesting that the central cholinergic system has an important role in learning and memory processes (1, 3, 4). Moreover, AF102B was found to compensate for cholinergic defects caused by AF64A or scopolamine treatment.

SDAT is characterized behaviorally by a general decline in cognitive function. Although several neurotransmitter systems are documented to be impaired (2), one of the most profound neurochemical changes observed is a marked presynaptic cholinergic hypofunction in selective brain regions including the hippocampus (1, 3). On the contrary, postsynaptic muscarinic receptors appear to be relatively unaffected (1, 5). Mash et al. (12) reported that cerebral cortex samples from SDAT patients exhibit reductions in the number of M₂ muscarinic receptors located presynaptically with no change in the number of M₁ receptors located postsynaptically. Pirenzepine, a selective M₁ antagonist, has been found to be very potent in impairing performance in a passive avoidance task, a Morris water maze and a T-maze in rodents, implying an important role of M₁ receptors in learning and memory processes (13–15). These findings suggest that selective M₁ agonists would be potentially useful therapeutic drugs for SDAT (3, 5).

That AF102B may be highly selective for M₁ receptors is suggested by the results of (a) displacement assays of [³H]QNB binding in the rat forebrain and cerebellum (16), (b) electrophysiological studies on rabbit superior cervical ganglia (17), and (c) neurochemical studies on presynaptic muscarinic autoreceptor and heteroreceptor regulating neurotransmit-

ter release (18). In the behavioral studies, we have already observed that AF102B significantly improves the performance of AF64A-rats in a T-maze and radial-arm maze tasks (7, 19). Taken together with the present results that AF102B ameliorates experimental amnesia (passive avoidance failure) in AF64A-rats and SCP-mice, AF102B deserves further examination for development as a possible therapeutic drug for SDAT.

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