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AF150(S): A New Functionally Selective M_1 Agonist Improves Cognitive Performance in Rats

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BRANDEIS, R., M. SAPIR, N. HAFIF, S. ABRAHAM, N. OZ, E. STEIN AND A. FISHER. *AF150(S): A new functionally selective M_1 agonist improves cognitive performance in rats.* PHARMACOL BIOCHEM BEHAV 51(4) 667-674, 1995.—This study was aimed at evaluating the ability of a new functionally selective partial M_1 agonist, AF150(S), to reverse cognitive impairments in rats. A memory deficits-induced animal model was used that involved AF64A (3 nmol/2 μl/side) bilaterally injected ICV. AF150(S) was administered PO. The pharmacodynamic profile of the compound was established and its general toxicity was evaluated. Animals were tested on three behavioral tasks: step-through passive avoidance, Morris water maze reference memory paradigm, and radial arm maze working memory paradigm. The sign-free dose of AF150(S) was > 40 mg/kg whereas the LD_{50} was > 500 mg/kg. In comparison, the effective dose in reversing performance impairments on the various tasks was much lower (0.5-5 mg/kg). The data suggest that AF150(S) possesses potential cognitive enhancement abilities, probably due to a specific increase of cholinergic function.

AF150(S) AF64A Passive avoidance Morris water maze Radial arm maze Reference memory Working memory Cognitive deficits Rats Alzheimer's disease

ALZHEIMER'S disease (AD) is one of the most prevalent disorders associated with aging (48), characterized by loss of memory and other cerebral functions, as well as brain structural and biochemical alterations in neuronal systems (25).

The degree of cognitive impairments in humans is highly correlated with degeneration or atrophy of the basal forebrain cholinergic projection system, which provides major cholinergic afferent inputs to both the hippocampal formation and the neocortical mantle (2,7,36). Decreases in choline acetyltransferase (ChAT) are most consistent and are strongly correlated with changes in memory (24,39). Acetylcholine (ACh) synthesis is also decreased in biopsy tissue from Alzheimer patients (44), and this decrease was shown to be significantly correlated with cognitive impairment (17). The cholinergic dysfunction in AD appears to be largely presynaptic with most authors reporting either normal levels of M_1 muscarinic receptors (8, 24,40) or only small decreases (28,43) or increases (47).

Pharmacological data also suggests a cholinergic involvement in learning and memory processes as muscarinic antagonists impair place learning in rats (21,23,37) and memory in humans (9,10). On the other hand, muscarinic agonists may enhance performance in both humans (30,41,42) and animals (33,34), probably by modulating neurotransmission via M_1 muscarinic receptors in the brain.

M_1-selective muscarinic agonists have been proposed as a promising treatment strategy in AD (18,28,38,51). Moreover, a centrally active agonist that exhibits M_1 selectivity (AF102B) has been synthesized by us in the past and was shown to be a cognitive enhancer in three memory disorders animal models: aged rats (5), ethylcholine aziridinium (AF64A)-injected rats (12,13), and C57BL/10 mice (49). Recently, a new conformationally rigid analogue of ACh, AF150(S)-[1-Methylpiperidine-4-spiro-(2'-methylthiazoline)], has been found by us to be a selective and efficacious M_1 agonist in rat cortex (15). Furthermore, in transfected cell cultures with m_1-m_5 AChRs, AF150(S) is both a functionally selective partial m_1 agonist and also activates distinct signalling pathways (15). This compound is probably limiting the repertoire of certain G-proteins, which may be activated by m_1 AChR, thus exhibiting selectivity not only at the receptor binding site but also along discrete signalling pathways. Similar results were reported by us recently with AF102B (19).

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For the purpose of evaluating the mnemonic characteristics of AF150(S) the AF64A-injected animal model was used. AF64A injected intracerebroventricularly (ICV) in rats induces a cholinergic hypofunction confined mainly to the hippocampus, as evident by a persistent decrease in both ChAT activity and high-affinity choline transport (HAChT) system (6,14,26). This cholinotoxicity mimics some of the cholinergic hypofunction reported in AD (14). We report here on the ability of AF150(S) to reverse AF64A-induced deficits in cognitive functioning in rats by assessing various learning and memory processes in three behavioral tasks: step-through passive avoidance (PA), Morris water maze (MWM), and eight-arm radial maze (RAM). All three tasks appear to be sensitive to lesions of the hippocampal formation and the nucleus basalis magnocellularis (NBM) (3,4,20,31,35,53) as well as to cholinergic manipulations (1,11,23,32,50,54).

METHOD

Pharmacodynamic Profile and General Toxicity

To evaluate the safety margin of AF150(S) animals were subjected to detailed observations of changes in general behavior, reflexes, autonomic effects, and mortality up to 24 h following treatment. One hour prior to the study AF150(S) was diluted with double distilled water (DDW). Male Sprague-Dawley rats, 200–300 g, were administered PO with five doses: 40, 50, 100, 200, and 500 mg/kg in a volume of 10 ml/kg. Treatment group size was four rats. The following parameters were observed: salivation, redness around the nose and mouth, chromodacryorrhea, sedation, ataxia, cyanosis, tremors, convulsions, hypothermia, opisthotonus, respiratory distress, diarrhea, gnowing, piloerection, and mortality.

AF64A Preparation

AF64A (10 mM) was prepared by alkaline hydrolysis of acetylethylcholine mustard HCl (12,16).

Surgical Preparation and Animals

Male Sprague-Dawley rats, 230–290 g, anesthesized with Equithesin (0.3 ml/100 g, IP) were injected bilaterally by stereotaxic application of AF64A (3 nmol/2 μl/side) or saline (2 μl) into the lateral cerebral ventricles (AP = −0.8; L = ±1.5 mm from bregma; and DV = −4.8 mm from skull surface). Infusions were made via a CMA 100 microinjection pump, through a 30-ga injection cannula, at a constant infusion rate of 0.25 μl/min. The cannula was left in place for 4 min after injection to allow diffusion of the solution into the ventricles.

Following surgery rats were housed, five per cage, in a temperature controlled environment (22 ± 1°C) with 12L : 12D cycle. Animals had ad lib access to food and drinking water except for the RAM task prior to which animals were food restricted until reaching approximately 80% of their free-feeding weight. Afterwards the rats received five to eight food pellets (Altromin, Lage) per day. Two days before training, the rats were fed with precision pellets (Bioserv, Inc. Frenchtown, NJ), which were later used for reinforcement in the maze.

Behavioral Tests

Passive avoidance.

Drug administration. At 4–6 weeks postoperation AF64A- and saline-injected rats were randomly subdivided into two subgroups (n = 9–10): Subgroup 1 was treated with AF150(S) and subgroup 2 was treated with DDW.

AF150(S) (1 mg/kg, PO), in a volume of 10 ml/kg, and DDW in the same volume were administered once, immediately after the shock, through a flexible feeding tube (2.7 mm o.d.).

Apparatus. Training and testing were carried out in a two-compartment box: a small illuminated compartment (21 × 16 × 22 cm) with a lamp (60 W) at a height of 25 cm above the top of the compartment, and a large dark compartment (37 × 23 × 22 cm). The two compartments were separated by a door (7 × 5 cm).

Training. In the acquisition trial, the rat was placed in the illuminated safe compartment; 60 s later the door was opened and the rat's latency to enter the dark compartment (to step through) was measured. Immediately following entry into the dark compartment, the rat was subjected to an inescapable scrambled foot shock applied to the grid floor (0.65 mA for 3 s). A cutoff point of 180 s was used for initial latency. After the acquisition trial the rat was returned to its home cage.

Testing. Retention of the passive avoidance task was measured 72 h later, by again placing the rat in the lighted front compartment and measuring the latency to enter the dark compartment. A cutoff point of 600 s was used for retention latency. Animals that failed to step through within 600 s were removed from the apparatus and a 600-s latency was recorded for them.

Morris Water Maze.

Drug administration. At 4 months postoperation each of the AF64A and saline groups of rats was randomly subdivided into four treatment groups (n = 9): subgroups 1–3 were treated with AF150S in doses of 0.5, 1, and 5 mg/kg, PO, in a volume of 10 ml/kg whereas subgroup 4 (control group) was treated with DDW in the same volume.

AF150(S) and DDW were administered once a day for 5 days, 60 min before testing, through a feeding tube, as noted in the PA section.

Apparatus. Rats were tested in a circular metal water maze (diameter: 1.4 m, height 50 cm), which was painted white and was filled to a height of 25 cm with water (26 ± 1°C) in which powedered milk was dissolved. A white metal platform (12 × 12 cm) covered by wire mesh was present inside the pool; its top surface was 20 mm below the surface of the water; thus the platform was invisible to a viewer inside the pool.

The pool surface was divided into four quadrants of equal area, NE, NW, SE, and SW. The platform was placed midway between the center and rim of the pool in any of the four quadrants. The maze was brightly lit and surrounded by well-lit salient objects, which were held constant throughout training. Performance in the maze was monitored by a tracking system consisting of an overhead video camera linked to a TV monitor and an image analyzer (CIS-2) coupled to a microcomputer (system designed and produced by Galai Laboratories, Ltd., Migdal HaEmek).

Habituation. Rats were placed in the pool with no platform for a 1-min habituation trial 72 h prior to the initiation of training.

Training. Each rat was trained for 4 consecutive days, four trials (one block) per day, in which the platform position remained constant and was located in the center of the SE quadrant of the pool. Within each block of four trials, each rat started at each of the starting locations, but the sequence of locations was randomly selected. A trial consisted of placing a rat by hand into the water, facing the wall of the pool at one of four starting locations, north, south, east, or west, around

the pool's perimeter. Prior to training, the rat was placed on the platform for 60 s. If, on a particular trial, a rat found the platform, it was permitted to remain on it for 60 s. A trial was terminated after 120 s if a rat failed to find the platform, and the rat was placed on the platform for 60 more s before starting the next trial. Path length (the distance travelled by the rat), escape latency (the time to find the platform), and speed (the swimming rate of the rat) were recorded on each trial by the monitoring system.

Transfer test. Three minutes follwing the last training trial (trial 16), the platform was entirely removed from the pool (a probe trial). In this trial (trial 17), the rat was placed into the water for a limited period (60 s), and its spatial bias was measured by recording the relative distribution of escape latency and path length over the four quadrants of the pool.

Reversal test. During trials 18–21, on the fifth day, the platform position was changed to the NW quadrant, opposite to the training quadrant. Thus, during reversal learning, the platform location was moved relative to the configuration of objects within the room, but the pool occupied the same place within the room throughout the entire experiment. Testing of the rats and measures taken were the same as in training.

Radial Arm Maze.

Drug administration. At 6–8 months postoperation each of the AF64A and saline groups of rats was subdivided into four treatment groups ($n = 9$) as was described for the MWM task. AF150(S) and DDW were administered, PO, once a day for 5 days, 60 min before testing.

Apparatus. Behavioral testing was conducted in an elevated (70 cm) eight-arm radial maze made of transparent PVC. The arms (75 cm long and 10 cm wide) extended from an octagonal central arena (40 cm wide). At the end of each arm a self-feeder was placed (45-mg pellet dispenser, Model 8000, Lafayette Instrument Company). Photocells installed within the maze and connected to a computer monitored correct and incorrect entries as well as the time spent in the task (System designed and produced by Mezada Corp., Ness-Ziona).

Pretraining. Before starting the actual test, rats were familiarized with the RAM. Pellets were scattered in the whole area of the maze. Rats were placed in the central arena, one at a time, always facing the same direction, and were permitted to run from arm to arm until visiting all eight arms or until 15 min had elapsed. Pretraining was continued for 2 days, one session per day.

Training. Each rat was placed in the central arena, the doors were opened, and the rats were permitted to run from arm to arm until eight pellets were collected or until 15 min had elapsed. Pellets were placed only at the end of the arms. All movements within the maze were recorded, elapsed time as well as correct and incorrect responses. Training continued for 3 days.

Testing. Testing period was carried out during the second week of the experiment, at which AF150(S) or DDW were administered. Otherwise the running procedure was the same as in training.

RESULTS

Pharmacodynamic Profile and General Toxicity

The effects of various doses of AF150(S) on general behavior are described below. The sign-free dose was estimated to be greater than 40 mg/kg. Redness around the nose and mouth appeared at the dose of 50 mg/kg, 30 min following drug administration in one animal only, and continued for 30 min. No other toxicological signs were observed at this dose. Salivation was shown at 100 mg/kg in two animals 30 min following drug administration and continued for 30 min. Chromodacryorrhea and sedation were first observed at 200 mg/kg. Chromodacryorrhea appeared in one animal 60 min after drug administration and continued for 60 min, whereas sedation appeared in all four animals 15 min after drug administration and continued for 225 min. Other effects like ataxia, cyanosis, tremors, convulsions, hypothermia, opisthotonos, and respiratory distress appeared at 500 mg/kg. The LD_{50} is estimated to be greater than 500 mg/kg; no mortality had been observed at this dose.

Passive Avoidance

Mean initial and retention latencies for each group are presented in Table 1. Significance of differences was determined by Mann–Whitney *U*-test.

No significant differences were found in the initial latency measures between the groups tested. However, the retention latency of AF64A-injected rats treated with DDW was significantly shorter (poorer memory) than that of saline-injected rats treated with DDW ($p < 0.05$). The retention latency of AF64A-injected rats treated with AF150(S) 1 mg/kg was significantly longer (better memory) than that of AF64A-injected rats treated with DDW ($p < 0.002$). No significant difference was found between the latencies of the two saline-injected groups [one treated with DDW, the other treated with AF150(S)].

Morris Water Maze

Training. For each rat, the path length, escape latency, and swimming speed of the four trials on each of the 4 training days were grouped into blocks (one block for each day). The scores of all three measures were analyzed by a three-way ANOVA ($2 \times 4 \times 4$) with one repeated variable (days) and two nonrepeated variables [injection—AF64A/saline, and treatment—three doses of AF150(S)]. Specific comparisons

TABLE 1

INITIAL AND RETENTION LATENCIES OF AF64A- AND SALINE-INJECTED RATS TREATED WITH AF150(S)

	Initial Latency		Retention Latency	
	AF150(S) (1 mg/kg)	Control	AF150(S) (1 mg/kg)	Control
AF64A	25.9 ± 6.0	26.6 ± 5.0	536.0 ± 43.3*	180.7 ± 41.3
Saline	31.6 ± 8.6	22.6 ± 7.5	556.7 ± 46.0	423.4 ± 75.3†

Values are mean ± SEM in seconds.

*$p < 0.002$, †$p < 0.05$, compared to AF64A-injected rats treated with DDW.

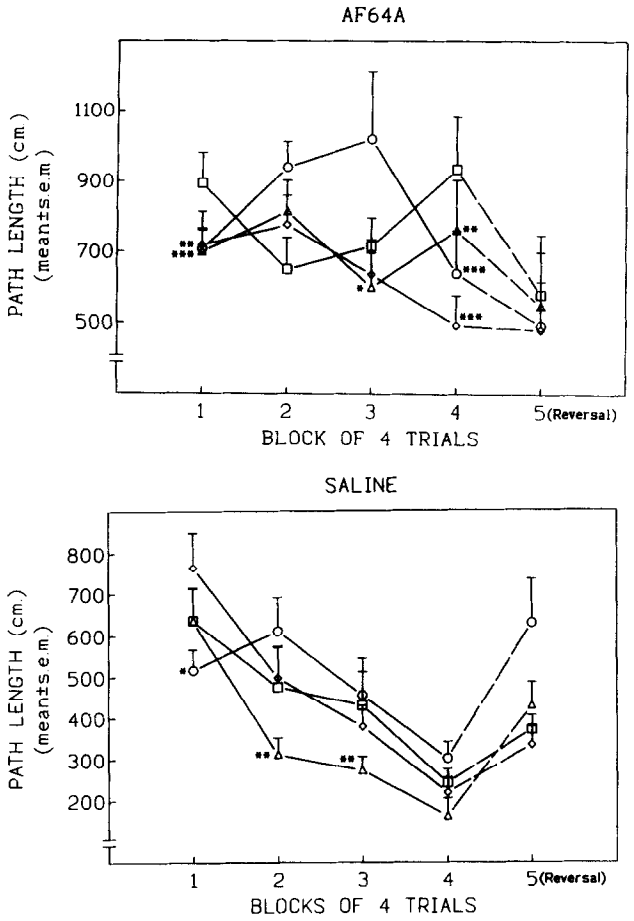

FIG. 1. Path length of AF64A- and saline-injected rats treated with AF150(S). Squares: DDW (10 ml/kg); triangles: 0.5 mg/kg; diamonds: 1 mg/kg; circles: 5 mg/kg. *$p < 0.02$, **$p < 0.01$, ***$p < 0.001$ compared to DDW.

were performed, using the simple main effects contrasts analysis (52), which is specifically suited for testing significant interactions.

Path length. Figure 1 presents the path length performance of AF64A- and saline-injected rats treated with various doses of AF150(S). AF64A-injected rats treated with DDW showed a significantly longer path length (indicating a worse RM performance) than saline-injected rats treated with DDW, during the four days of training, $F(3, 192) = 6.32$, $p < 0.001$. AF150(S) treatment positively affected the training performance of AF64A-injected rats [the interaction treatment × days was found significant, $F(9, 192) = 2.83$, $p < 0.01$]. Specifically, a dose of 0.5 mg/kg improved the path length performance of AF64A-injected rats during the first ($p < 0.001$), third ($p < 0.02$), and fourth ($p < 0.01$) days of training. The doses of 1 and 5 mg/kg resulted in a similar improving effect during the first ($p < 0.01$) and fourth ($p < 0.001$) days. On the third day a deterioration in the performance of AF64A-injected rats was shown by the dose of 5 mg/kg.

AF150(S) partially improved the performance of saline-injected rats at the doses of 0.5 mg/kg (days 2-3, $p < 0.01$) and 5 mg/kg (day 1, $p < 0.02$). However in some days deterioration in performance was observed: day 2, 5 mg/kg ($p < 0.01$); day 1, 1 mg/kg ($p < 0.02$).

A characteristic computer depiction of the path length traveled by AF64A- and saline-injected rats treated with AF150(S) during training is shown in Fig. 2.

Escape latency. All AF64A-injected rats showed a significantly longer escape latency (49.0 ± 2.0 s, indicating a worse RM performance) than all saline-injected rats (27.6 ± 1.5 s), $F(1, 64) = 67.45$, $p < 0.001$. AF150(S) treatment had a partial positive effect on training performance of both AF64A- and saline-injected rats [the interaction treatment × days was found significant, $F(9, 192) = 2.21$, $p < 0.05$]. The escape latencies of rats treated with the dose of 0.5 (day 3) and 1 mg/kg (day 4) were significantly shorter ($p < 0.05$ and $p < 0.02$, respectively) than those of rats treated with DDW. However, the higher dose (5 mg/kg) resulted in a decrease in performance on day 2 ($p < 0.01$).

Swimming speed. AF150(S) (0.5, 1, and 5 mg/kg) significantly improved the swimming speed of both AF64A- and saline-injected rats on day 3, $F(9, 192) = 5.69$, $p < 0.001$. However, the higher dose (5 mg/kg) significantly decreased the swimming speed on the second day of training ($p < 0.01$). No correlation was found between path length or escape latency and swimming speed parameters.

Transfer test. The path length and escape latency for the transfer trial (trial No. 17) were analyzed by a three-way ANOVA (2 × 4 × 4) with one repeated variable (quadrant in the pool) and two nonrepeated variables [injection—AF64A/saline, and treatment—three doses of AF150(S)].

In both parameters, path length and escape latency, all saline-injected rats showed a spatial bias in the transfer test. They spent significantly more time and swam a significantly longer distance in the training quadrant relative to the three other quadrants of the pool [$F(3, 192) = 24.91$, $p < 0.001$ and $F(3, 192) = 25.99$, $p < 0.001$, for the two injection × quadrant interactions, respectively]. On the other hand, AF64A-injected rats treated with DDW did not show any spatial bias on this test; they spent an equal time in each of the four quadrants of the pool and the total distance traveled (Fig. 3) was also equal in each of the quadrants. However, AF64A-injected rats treated with AF150(S) showed a partial bias [the treatment × quadrant interactions were found significant, $F(9, 192) = 3.35$, $p < 0.001$ and $F(9, 192) = 2.97$, $p < 0.001$, for path length and escape latency, respectively]. They spent more time and swam a longer distance in quadrant No. 1 relative to quadrants No. 3 and 4, but not relative to quadrant No. 2.

Reversal test. For each rat the path length, escape latency, and swimming speed of trials 18-21 on the fifth day were grouped into one block. The scores were analyzed by a two-way ANOVA (2 × 4) with two variables [injection—AF64A/saline, and treatment—three doses of AF150(S)]. No significant differences were found between any of the groups tested in this test.

Radial Arm Maze

An analysis was made to evaluate the effect of AF150(S) during testing period compared to training period, which was used as a performance baseline. For this purpose a three-way ANOVA (2 × 4 × 2) with one repeated variable (period of time) and two nonrepeated variables [injection—AF64A/saline, and treatment—three doses of AF150(S)] was conducted.

Correct choices. The interaction injection × treatment ×

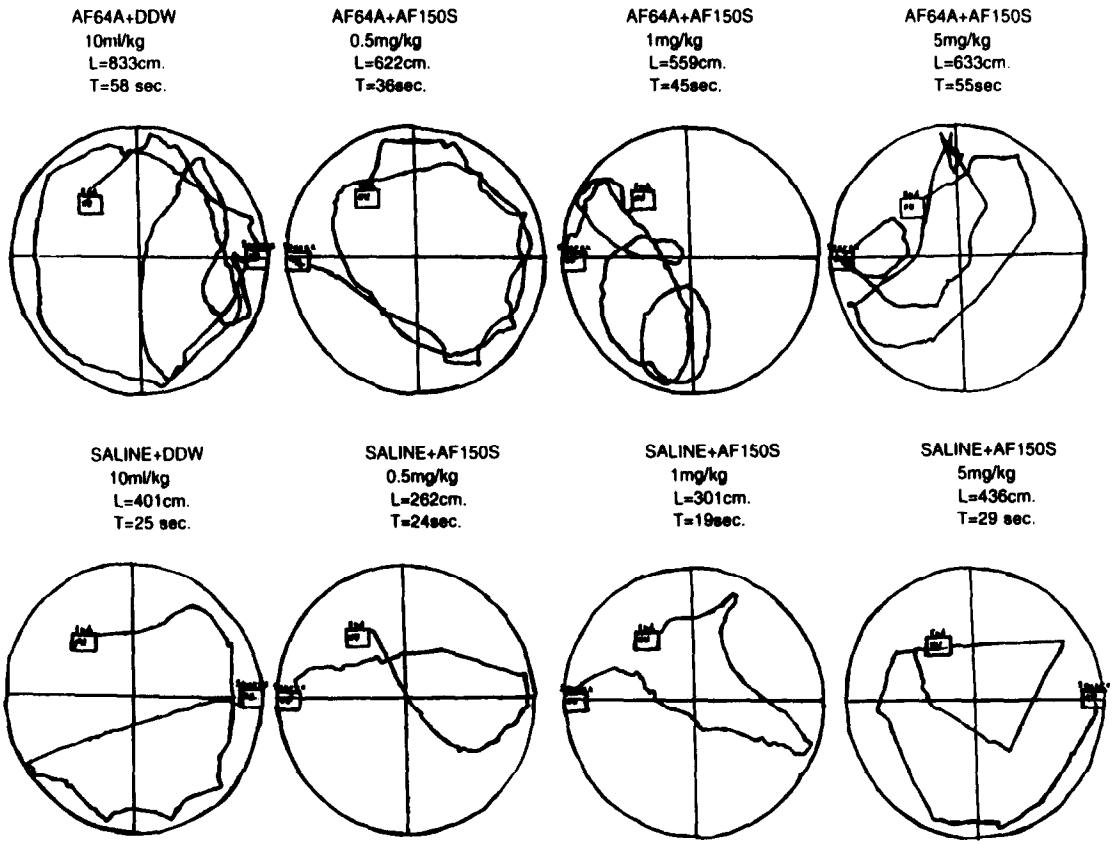

FIG. 2. A characteristic computer depiction of the path length travelled by AF64A- and saline-injected rats treated with AF150(S).

period of time was found significant, $F(3, 64) = 2.7$, $p < 0.05$. Subsequent contrasts analysis showed that the number of correct choices out of the first eight entries of AF64A-injected rats treated with AF150(S) (1 mg/kg) significantly ($p < 0.001$) increased during drug administration compared to baseline (Fig. 4). However, the number of correct choices of AF64A-AF150(S) 0.5 mg/kg rats significantly decreased ($p < 0.01$) during drug administration. In contrast, performance of AF64A-DDW rats did not change during the testing period. In addition, the performance of saline-injected rats treated with DDW improved during the testing period ($p < 0.001$).

Similar results were found when groups were compared only during the week of drug administration; AF150(S) 1 mg/kg significantly improved performance of AF64A-injected rats ($p < 0.05$, compared to AF64A-injected rats treated with DDW) whereas the dose of 0.5 mg/kg resulted in a deterioration in performance ($p < 0.05$). Saline-injected rats treated with 1 or 5 mg/kg also showed a decrease in performance during testing compared to rats treated with DDW ($p < 0.01$ and $p < 0.001$, respectively). It should be noted that a significant difference ($p < 0.001$) was found in the number of correct choices between AF64A- and saline-injected rats treated with DDW.

Total errors and total time. The number of errors of all AF64A-injected rats (7.9 ± 0.5) was significantly larger than that of all saline-injected rats (3.1 ± 0.3), $F(1, 64) = 68.24$, $p < 0.001$. No effect of AF150(S) was revealed in this parameter. Total time was not affected by either injection or treatment.

DISCUSSION

AD is the most common acquired progressive brain syndrome. It is associated with global deterioration of cognitive functioning and results in severe social impairment. The cholinergic deficit demonstrated in AD patients raises the possibility that pharmacological enhancement of central cholinergic function might benefit these patients. One such strategy is to enhance the cholinergic activity of neurons by pharmacological intervention at the receptor site.

AF64A-injected rats demonstrated in our study a clear impairment in learning and memory processes: remote memory in the PA task, acquisition and recent spatial reference memory (RM) in the MWM task, and spatial working memory (WM) in the RAM task were all impaired. AF150(S) significantly attenuated these impairments. The improvement of cognitive functioning was more pronounced during acquisition and retention. Reversal learning (in MWM task), which was defined as an expression of the ability to shift strategies to task demands (45), was not impaired by AF64A. Therefore, no positive effects of AF150(S) could be demonstrated in this test.

A dose-response effect of AF150(S) was demonstrated by the significant improvement of AF64A-induced cognitive defi-

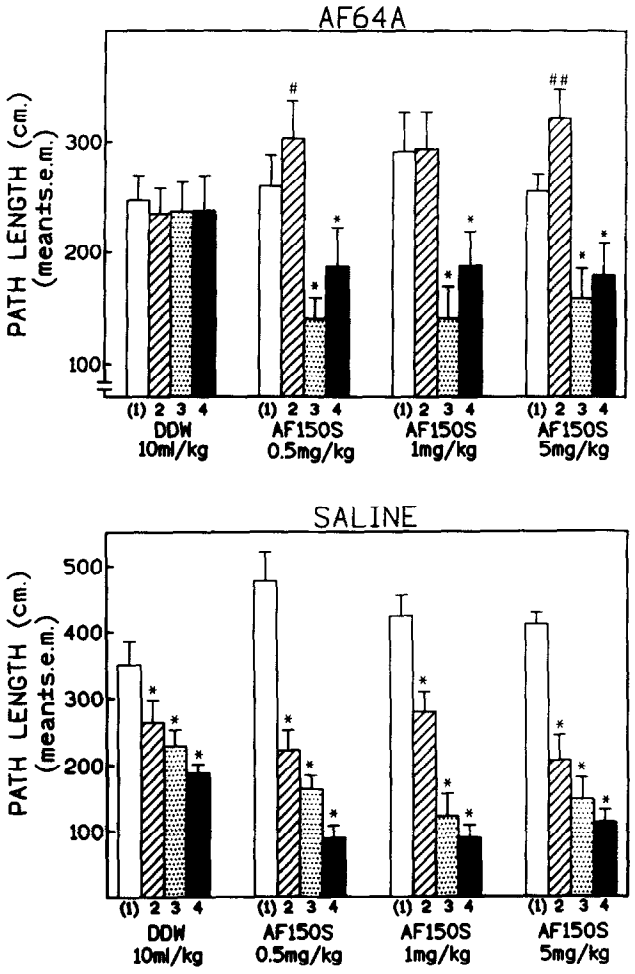

FIG. 3. Distribution of path length during transfer trial for AF64A- and saline-injected rats treated with AF150(S). *$p < 0.001$ compared to quadrant No. 1 in the respective group. #$p < 0.01$, ##$p < 0.005$ compared to quadrant No. 1, opposite direction.

cits, shown in the acquisition phase of the MWM task. The dose of 0.5 mg/kg improved performance during 3 training days whereas the doses of 1 and 5 mg/kg had a similar effect only during 2 training days [in this respect it should be noted that during the second training day performance was improved in AF64A-injected rats; therefore, the effect of AF150(S) on their performance could not be appropriately evaluated on that day]. During transfer trial, a significant partial improvement of memory deficits was demonstrated equally well at the three doses tested: 0.5, 1, and 5 mg/kg. Because the probe trial is the foremost procedure in the MWM task, providing measures that quantify the strength and accuracy of the original learning (29), it seems that all three doses had a positive, albeit partial, beneficial effect on the strategy used by the AF64A-AF150(S)-treated rats to locate the platform.

A dose-response effect was also observed in the RAM. WM was improved in AF64A-injected rats following treatment with the dose of 1 mg/kg. The dose of 0.5 mg/kg decreased performance whereas no effect was found following the administration of 5 mg/kg. These results are in accordance with the view that there is a high degree of concordance between the neurotransmitter systems implicated in place learning in the MWM and those implicated in the appetitively motivated RAM (27).

The results of this study indicate the broad-range effects of AF150(S). Thus, reference memory and working memory deficits were attenuated following drug administration. Furthermore, passive avoidance memory deficit was also significantly improved. In this respect it seems that AF150(S) has a positive effect on both declarative (explicit) and nondeclarative (implicit) memories (46).

Nonspecific motor coordination effects could explain neither the behavioral deficits of AF64A-injected rats nor the improving effects of AF150(S), because no consistent effects were demonstrated in the swimming ability of the rats in the MWM during testing. These findings, together with the results of the probe trial and the lack of effect in the total time measure of the RAM, strongly suggest that the pharmacological effects of AF150(S) are specific for learning and memory processes (29). Consistent with this view is the finding that AF150(S) attenuated memory deficits in three differently motivated behavioral tests. This rules out subtle effects of AF150(S) on motivational processes required to permit the animal "knowledge" to be manifested in behavior.

AF150(S), at the behavioral effective doses, has no undesirable toxicological effects. The sign-free dose was >40 mg/kg whereas the effective doses in attenuating AF64A-induced performance impairments ranged between 0.5 and 5 mg/kg (depending on test). These findings indicate that AF150(S) has a wide safety margin of approximately 10–100, resulting in a fairly wide therapeutic range.

Our results support the assertion of the "cholinergic hypothesis" (2); namely, that proper enhancement of cholinergic function may significantly reduce the severity of age-related cognitive losses. The results of previous studies with cholinergic agents were very subtle for diverse reasons (5). However, M_1-specific agonists may be more efficacious in improving overall cholinergic function and reducing cognitive disturbances associated with AD (5,28,38).

AF150(S) is relatively selective m_1, as evidenced both by its preference for M_1 receptors in the rat cortex and its functional selectivity towards m_1 receptors in cell cultures (15). AF150(S) activated m_1 AChR-mediated signalling in cell cultures such as increase in phosphoinositides turnover, increase in arachidonic acid release, and increase in intracellular Ca^{2+}. However, unlike carbachol or acetylcholine, AF150(S) did not have any agonistic effects on cAMP levels in the same cell cultures

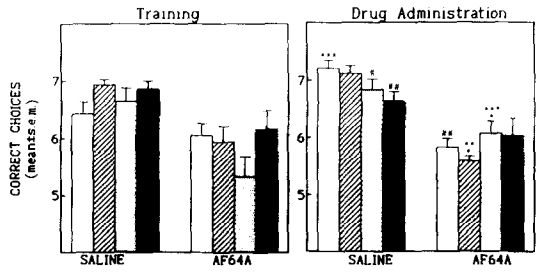

FIG. 4. Correct choices of AF64A- and saline-injected rats treated with AF150(S). Open bars: DDW, hatched bars: 0.5mg/kg, dotted bars: 1 mg/kg, filled bars: 5 mg/kg. *$p < 0.05$ compared to AF64A + DDW. **$p < 0.01$, ***$p < 0.001$ compared to respective group in training. #$p < 0.01$, ##$p < 0.001$ compared to saline + DDW.

(15). AF150(S), being a rigid analogue of ACh, might limit the repertoire of G-protein, which the m_1 receptor subtype may activate.

Notably, mRNA for $G_s\alpha$ and m_1 AChR were elevated in postmortem brain tissues of AD patients (22), and an elevation in the ratio of G_s/G was reported in aged human brains (55).

It can be speculated that in AD there is an increased sensitivity of m_1 AChR-mediated activation of adenylyl cyclase in these situations. It is thus possible that the m_1-selective agonists designed for the treatment of AD should not stimulate adenylate cyclase in these situations (19). AF150(S) might fulfil such a condition.

In conclusion, results shown here support the concept that a partial M_1 agonist with both greater specificity for a receptor subclass and greater selectivity for certain signal transduction pathways may provide a valuable tool for treating deficits in cognitive functioning associated with AD.

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