Pharmacology Biochemistry & Behavior, Vol. 36, pp. 89–95. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Reversal of Age-Related Cognitive Impairments by an M1 Cholinergic Agonist, AF102B

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Received 13 March 1989

BRANDEIS, R., S. DACHIR, M. SAPIR, A. LEVY AND A. FISHER. *Reversal of age-related cognitive impairments by an M1 cholinergic agonist, AF102B.* PHARMACOL BIOCHEM BEHAV **36**(1) 89–95, 1990.—This study examined the effect of a specific M1 cholinergic agonist, AF102B, on place learning of aged and young rats. Spatial reference memory was tested in the Morris Water Maze task, while spatial working memory was tested on an 8-arm radial maze. Both memory functions were impaired in aged rats compared to young animals. However, the administration of AF102B significantly reduced the age-related cognitive impairments observed in both tasks. This data supports the assertion of the "cholinergic hypothesis," namely that specific enhancement of cholinergic function may reverse geriatric cognitive deficits.

Aging M1 agonist AF102B Morris water maze Radial arm maze

AGING is associated with cognitive behavioral impairments, as well as brain structural and biochemical alterations in discrete neuronal subsystems. Both aged rats and aged humans can exhibit severe deficits in cognitive abilities (1, 2, 4).

Recent studies (4, 10, 40) have shown that 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. Decline in cerebral cholinergic function is seen also in aging rodents (4, 23, 29). Since the age-related decline in learning and memory is reminiscent of the deficits seen after lesions to the septo-hippocampal system in rats, decrements or degenerative changes in the septo-hippocampal cholinergic projection system may play a major role in the development of such deficits. Pharmacological data also suggests a cholinergic involvement in learning in rats as muscarinic antagonists impair place learning (6, 8, 26, 28, 30, 41, 46), probably by blocking neurotransmission at M1 muscarinic receptors in the brain (25,28).

Recently, M1 selective muscarinic agonists have been proposed as a promising treatment strategy in Alzheimer's disease (AD) (25, 28, 31, 42, 50), the major type of dementia among the elderly. Conformationally rigid analogs of acetylcholine (ACh) may provide such selective agonists. Such a rigid analog of ACh, (±)cis-2-methyl-spiro-(1,3-oxathiolane-5,3′)-quinuclidine (AF102B), has been recently synthetized by us (16–20). The design of this drug was based on the premise that utmost rigidity would limit the ability of the ligand to adapt to subtle differences in receptor structure, or its microenvironment and thus provide selectivity towards a certain population of the muscarinic receptors. It was shown by us and others, that AF102B is a centrally active agonist which exhibits M1 selectivity (16–20, 33). Moreover, in ethylcholine aziridinium (AF64A)-injected rats, low doses of AF102B reversed cognitive impairments shown in three learning and memory tasks, namely one-trial, step-through passive-avoidance (PA), 8-arm radial maze (RAM) and Morris Water Maze (MWM).

In the present study, we examined the effects of AF102B administration on place learning in two spatial memory tasks, MWM and RAM, in aged rats. Both tasks appear to be particularly sensitive to lesions of the hippocampal formation and the nucleus basalis magnocellularis (NBM) (7, 24, 32, 35, 37–39, 45, 49), as well as to the aging process (3, 9, 11, 13, 14, 21, 43, 47). The first task, the MWM, assesses spatial learning abilities in a reference memory (RM) procedure which is free from working memory (WM) components (24,28), as well as from local olfactory visual or kinaesthetic cues (34). The other paradigm, the RAM, measures spatial WM ability, which is considered the closest analogy to recent memory in man (37). This task is also free of local cues but with recourse to food deprivation.

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METHOD

SUBJECTS

Old male Wistar rats (18–19 months old, in the MWM task and 26–27 months old in the RAM task, 500–730 g), were housed two (MWM) or one (RAM) per cage, in a temperature-controlled environment (22 ± 1°C) with 12-hr normal light/dark cycle. Young male Wistar rats (3 months old, 300–350 g), were housed under the same conditions. The MWM task animals had ad lib access to food and drinking water. The RAM task animals were food-restricted until reaching approximately 75% of their free feeding weight. Afterwards the rats received 6 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. The rats had free access to water. Behavioral testing was carried out between 08.00 and 14.00 hr, five days a week.

BEHAVIORAL TESTS

MWM

Drug administration. Each of the young and old groups of rats was subdivided into two treatment subgroups (n = 10): Subgroup 1 was treated with AF102B and subgroup 2 was treated with saline. AF102B (1 mg/kg, IP) in a volume of 1 ml/kg and saline were administered once a day for 5 days, 30 min before testing.

Apparatus. Rats were trained and tested in a white circular metal water maze measuring 140 cm (dia.) × 50 cm (height) and filled to a depth of 25 cm with water (26 ± 1°C). The maze was brightly lit and surrounded by well lit, salient objects. 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 (8MZHz-IBM AT) (system designed and produced by Galai Laboratories, Ltd., Migdal Ha-Emek).

The pool surface was divided into 4 quadrants of equal area, NE, NW, SE and SW. During place navigation training, escape was provided by a wooden platform (12 × 12 cm) covered by a wire mesh and painted white, which stood 2 cm below the water surface. Addition of dried milk powder rendered the platform invisible at water level. The platform was placed midway between the center and rim of the pool in any one of the 4 quadrants.

Procedure.

Habituation. Seventy-two hr prior to the start of training, rats were placed in the pool with no platform for a one-minute habituation trial.

Training. Each rat was given four training trials per day on four consecutive days. Starting locations were randomly varied except that in each day, the rat had to start at least once from each starting point. A trial started when the rat, held facing the pool wall, was immersed in the water. It was then allowed 120 sec in which to search for the platform and if it failed to escape within this time period, it was placed on the platform. Irrespective of whether the rat found the platform or was placed on it, it remained there for 60 sec. The rat was then removed and given the other three trials and finally returned to its cage. For each rat, the platform position remained constant throughout the four training days. Escape latency (the time to find the platform), path length (the distance travelled by the rat) and speed (the swimming rate of the rats) were recorded on each trial by the monitoring system.

Reversal test. Twenty-four hours after the final training trial (day 5), the "reversal ability" of the rat was tested. Rats were tested as in the training session, except that the platform position was changed to the quadrant opposite to the training quadrant. Reversal testing was continued for four trials. Measures taken were the same as in the training period.

RAM

Drug administration. Each of the young and old groups of rats was subdivided into two treatment subgroups (n = 10): subgroup 1 was treated with AF102B and subgroup 2 was treated with saline. AF102B (1 mg/kg, IP) and saline in a volume of 1 ml/kg were administered once a day for two weeks, 5 days a week, two minutes before testing.

Apparatus. Behavioral testing was conducted in an elevated (70 cm) 8-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).

Procedure.

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 8 arms or until 15 min had elapsed. Pretraining was continued for three days, one session per day.

Training. Each rat was placed in the central arena, immediately following injection of AF102B or saline. Two minutes postinjection, the doors were opened and the rats were permitted to run from arm to arm until 8 pellets were collected or until 15 min had elapsed. All movements within the maze were recorded, elapsed time as well as correct and incorrect responses. Training continued for two weeks, 5 days a week.

RESULTS

MWM

Escape Latency

For each rat, the escape latencies of the four trials in each day were grouped into blocks (one block for each day).

The escape latency scores were analyzed by a three-way ANOVA (2 × 2 × 4) with one repeated variable (days) and two nonrepeated variables (age-old/young and treatment-AF102B/saline). Figure 1 presents the mean ± S.E.M. of the escape latency measures (the scores of one animal from each of the old subgroups were excluded because these animals were floating rather than swimming). Because of the statistical requirements, one score of each of the young subgroups was also excluded randomly.

Old rats showed significantly larger escape latencies (indicating a worse RM performance) than the young rats, $F(1,32) = 43.38$, $p<0.001$. The results indicated also an interaction between age and treatment, $F(1,32) = 5.53$, $p<0.05$. The escape latencies of the old rats treated with AF102B was significantly shorter than that of the old rats treated with saline during the 4 days of training and also during the reversal test ($p<0.01$, by a simple main effects contrasts analysis). There was no significant difference between the escape latencies of the young rats treated with AF102B and young rats treated with saline during both training and reversal, perhaps because of a "ceiling" effect.

A significant general effect of training was shown, $F(4,128) =$

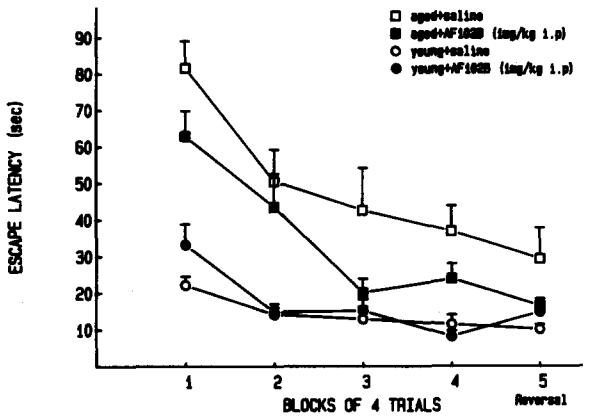

FIG. 1. Escape latency of old and young rats in presence of AF102B and saline.

30.98, $p<0.001$. The escape latencies decreased during the first three days of testing and then became stable. The results indicated also an interaction between age and days of learning, $F(4,128)=8.25$, $p<0.001$. More specifically, the escape latencies of the old rats decreased during the first three days of testing and then the escape latencies stabilized, while the escape latencies of the young rats decreased already during the first two days and only then became stable.

The standard deviation of the old rats treated with AF102B (9.65) was significantly smaller than that of the old rats treated with saline (18.66), $F(9,9)=3.73$, $p<0.05$.

Path Length

The measure of path length was added in this study in order to test whether rats learned to use a true mapping strategy instead of acquiring a nonspatial strategy which might help them to find the hidden platform.

The scores of this measure were statistically analyzed as described for the escape latency scores. Figure 2 presents the means ± S.E.M. of the path length measures. (The scores of one animal from each of the old subgroups were excluded because of

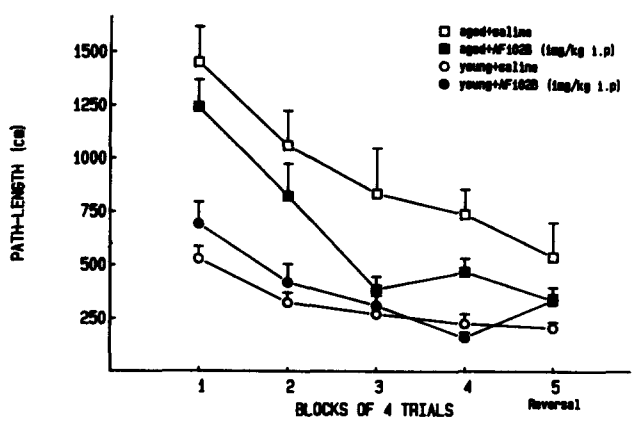

FIG. 2. Distance run by old and young rats in presence of AF102B and saline.

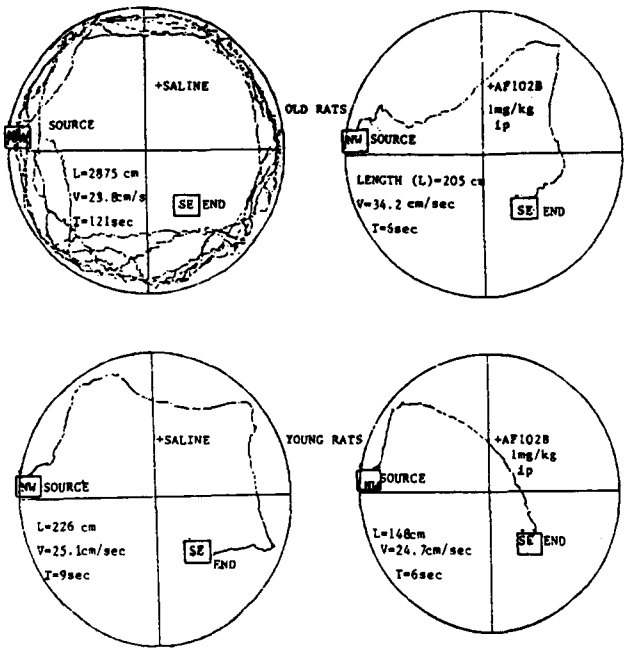

FIG. 3. A characteristic computer depiction of the path length of old and young rats in presence of AF102B and saline.

the same reasons described in the escape latency chapter.)

Old rats showed a significantly longer path length (indicating a worse RM performance than the young rats, $F(1,32)=38.41$, $p<0.001$. A statistically significant interaction between age and treatment, $F(1,32)=5.70$, $p<0.025$, showed that in the old rats AF102B decreased the length of the path during the 4 days of training and also during the reversal test ($p<0.01$, by simple main effects contrasts analysis), while in the young rats this effect was not found, maybe because of a "ceiling" effect. A significant general effect of learning had been shown, $F(4,128)=33.38$, $p<0.001$. The path length decreased during the first three training days and then stabilized. An interaction between age and days of learning was found, $F(4,128)=6.14$, $p<0.001$. The path length of the old rats improved during the first three days ($p<0.05$) and then stabilized, while in the young rats this improvement was shown already in the first two days.

The standard deviation of the old rats treated with AF102B (149.20) was significantly smaller than that of the old rats treated with saline (370.15), $F(9,9)=6.15$, $p<0.01$.

A characteristic computer depiction of the path length travelled by the old and young rats in presence and absence of AF102B or saline is shown in Fig. 3.

Swimming Speed

The parameter of swimming speed (cm/sec) is intended to measure the swimming ability of the rats. This motor coordination measure was introduced in order to separate the specific memory impairment from the more generalized motor changes associated with aging.

The scores of this measure were statistically analyzed as described for the escape latency and the path length scores. Figure 4 presents the means ± S.E.M. of the swimming speed measures.

An interaction was found between age and days of training, $F(4,144)=2.65$, $p<0.05$. In the first two days of training, the

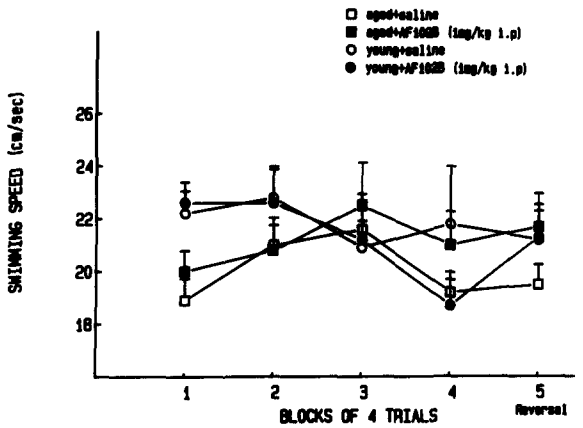

FIG. 4. Swimming speed of old and young rats under AF102B and saline treatments.

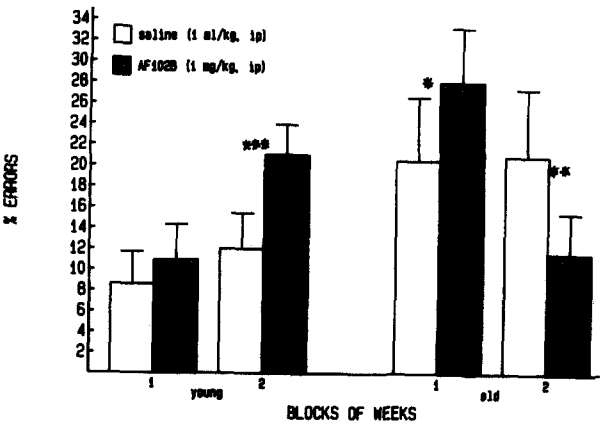

FIG. 6. Percent errors of old and young rats treated with AF102B or saline.

swimming speed of the old rats was significantly slower than that of the young rats. However, such a difference was not found later on.

The administration of the drug (AF102B) had no effect on the swimming speed of the rats.

Radial Arm Maze

In the following analyses, the scores of two animals from each of the old subgroups were excluded because these animals were extremely slow in adapting to the maze. Because of the statistical requirements, two scores of each of the young subgroups were also excluded randomly.

Correct Choices

An overall significant difference was found in the average number of correct choices out of the first eight entries between the old and young rats, by a three-way ANOVA with one repeated measures variable, $F(1,28)=5.5$, $p<0.05$. Young rats performed significantly better than old rats (7.4 ± 0.09 vs. 6.7 ± 0.19 correct choices, see Fig. 5). A three-variable interaction between group (old/young) × treatment (*AF102B*/saline) × trial (week I/week II) was also found.

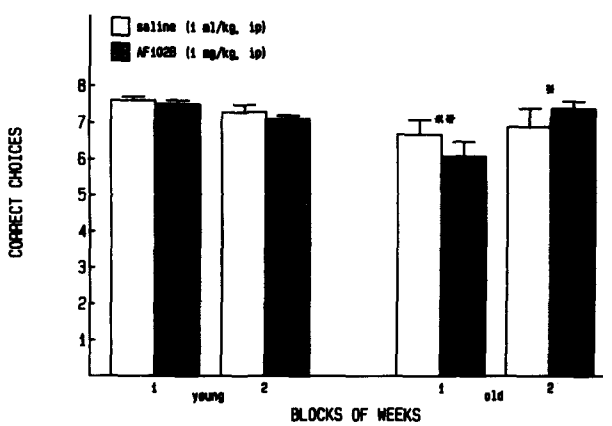

FIG. 5. Correct choices of old and young rats treated with AF102B or saline.

A simple main effect contrasts analysis revealed that during the first week, the AF102B-treated old rats made significantly less correct choices than the saline-treated old rats (6.1 ± 0.4 vs. 6.7 ± 0.4, $p<0.01$). However, during the second week, the situation was reversed: the AF102B-treated old rats made significantly more correct choices than the saline-treated old rats (7.4 ± 0.2 vs. 6.9 ± 0.5, $p<0.05$). At the same time, AF102B-treated old rats improved their performance significantly from the first to the second week (6.1 ± 0.4 vs. 7.4 ± 0.2 correct choices, $p<0.001$) while the saline-treated old rats did not exhibit any improvement (6.7 ± 0.4 vs. 6.9 ± 0.5).

No significant differences were found between the AF102B-treated young rats and the saline-treated young rats during the first or the second week of the experiment. However, there was a significant impairment of performance of AF102B-treated young rats during the second week of the experiment where they made less correct choices than during the first week [7.5 ± 0.1 (first week) vs. 7.1 ± 0.1 (second week), $p<0.02$]. The saline-treated rats showed no change in performance.

Percent Errors

A comparison of the percent errors (total number of incorrect entries as a percentage of the total number of entries) between the four treatment subgroups (old/young, AF102B/saline), over the two weeks, by a three-way ANOVA with one repeated variable, showed an interaction between group × treatment × trial, $F(1,28)=4.3$, $p<0.05$ (see Fig. 6). A simple main effect contrasts' analysis indicated that during the first week the AF102B-treated old rats made significantly more errors than the saline-treated old rats (28.0 ± 5.2 vs. 20.5 ± 6.1 percent errors, $p<0.05$). However, during the second week, the situation reversed: The AF102B-treated old rats performed significantly better than the saline-treated old rats (11.6 ± 3.9 vs. 20.9 ± 6.5 percent errors, $p<0.02$). AF102B-treated old rats improved their performance significantly from the first week to the second (28.0 ± 5.2 vs. 11.6 ± 3.9 percent errors, $p<0.001$), while the saline-treated old rats showed no improvement. A significant impairment of performance of AF102B-treated young rats was found during the second week when compared to the first week (10.9 ± 3.4 vs. 21.0 ± 2.9, $p<0.01$), while saline treatment had no effect on the rats' performance. Similarly, no difference was found between AF102B-treated young rats and saline-treated young rats during the first week while during the second week the AF102B-treated young rats made significantly more errors than the control saline-treated

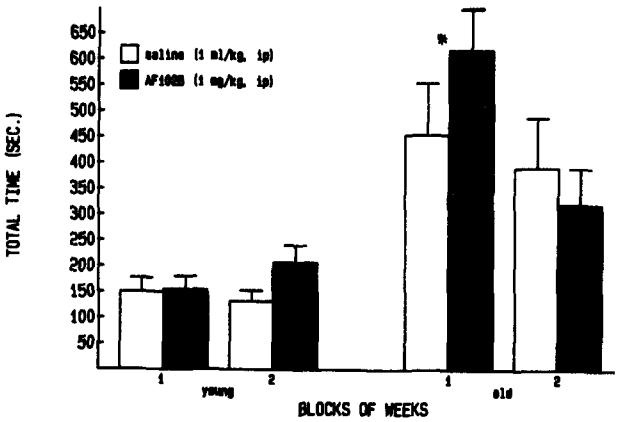

FIG. 7. Total time of old and young rats treated with AF102B or saline.

young rats (21.0 ± 2.9 vs. 12.0 ± 3.4, $p<0.02$).

Total Time

An overall significant difference was found in total performance time between old and young rats, by a three-way ANOVA with one repeated variable, $F(1,28) = 22.61$, $p<0.001$. Young rats completed their tasks significantly faster than the old rats (161.2 ± 14.7 vs. 447.5 ± 45.07 sec) (see Fig. 7).

An interaction between group × treatment × trial was found in this measure too. A simple main effect contrasts' analysis revealed that during the first week of the experiment, the AF102B-treated old rats needed more time to conclude their task than saline-treated old rats (619.1 ± 77.9 vs. 456.6 ± 99.7 sec, $p<0.01$). However, during the second week, no differences were detected between the two groups. Both subgroups, AF102B-treated old rats and saline-treated old rats improved their performance during the second week. They needed significantly less time to conclude their task as compared to the first week (321.4 ± 70.0 vs. 619.1 ± 77.9 sec, $p<0.001$ for the AF102B-treated subgroup and 393.0 ± 96.3 vs. 456.6 ± 99.7 sec, $p<0.05$ for the saline-treated subgroup). No differences were found between the AF102B-treated young rats and the saline-treated young rats during the first or the second week. Also, no differences were found between the first and the second week within each treatment group.

DISCUSSION

Cognitive disturbances represent one of the primary neuropsychiatric complaints of the elderly (48). Of the many approaches to developing animal models of age-related memory disturbances, one that is gaining increasing popularity involves the use of aged animals (5,22). This model is based on the underlying premise that the more an animal model accurately mimics the etiology and symptomatology of the aged human brain and behavior, the greater will be its predictive value in applications, such as drug testing (5). From this point of view, it seems that our chosen aged animal model supports the criteria for developing behavioral models of aging as well as for evaluating pharmacological treatments.

Old rats demonstrated in this study a decrease in learning and memory abilities; spatial RM in the MWM task and spatial WM in the RAM task were both impaired. AF102B significantly reversed the learning and memory impairments observed in both tasks. Moreover, AF102B also improved the reversal learning impairments of the old rats, as shown very clearly by both measures in the reversal test of the MWM. The result reveals the potential effect of AF102B on the ability to adopt and shift strategies in relation to task demands, a cognitive function which is impaired both in aged rats and in AD. Such a "shifting of set" behavior was shown before to be very sensitive to cholinergic manipulations (44).

The old rats varied tremendously with respect to their memory disturbance, as was shown by their extremely large standard deviation in the MWM. These individual differences in the age-related decline of spatial memory have also been demonstrated in other studies (21), and is an important feature of an animal model of age-related memory dysfunction, since it mimics the phenomenology of the human disorder very closely (15). The marked individual variations in spatial memory capacity may reflect various degrees of impairment in hippocampal synaptic connectivity in animals of the same chronologically advanced age (15,29). This was demonstrated by the partial decrease in the number of perforated axospinous synapses in the dentate gyrus and CA1 (15) and in the area of cells and number of cells in CA3 hippocampal subfield (29).

The experimental findings reported in this study demonstrate that the degree of individual differences in spatial memory deficit in aged rats, can be markedly reduced by subchronic (one to two weeks) treatment with AF102B, an M1 selective agonist. No doubt that this should be one of the features of a future efficient treatment for human age-related cognitive impairments.

Motor coordination effects could neither explain the behavioral deficits of the old rats nor the beneficial effects of AF102B expressed in both tasks, since no such effects were demonstrated in the swimming ability test of the MWM task.

One of our findings that needs to to be explained was that the treatment by AF102B at first caused a decrease in performance of old rats (first week) in the RAM, followed by an improvement during the second week. In young animals, the decrease in performance appeared during the second week. A possible explanation is that in young rats, an increase in release of acetylcholine (ACh) following treatment with the cholinergic agonist AF102B is compensated by down regulation of the cholinergic receptors. After two weeks, the down regulation cannot compensate for the consistent increase in ACh and decrease in performance occurs. In older animals, the capability to down (or up) regulate the density of receptors is reduced (36) and therefore increase in ACh causes an almost immediate decrease in performance. However, it seems that after a longer period of time (two weeks), a different mechanism is affected by which the increase in ACh causes an improvement in performance. This mechanism would probably be related to receptor function (as compared to receptor density), for instance, stimulation of the receptor-effector system (36). It is reasonable to assume that this system would be impaired in old animals and therefore prone to improvement, more so than in young animals.

It is interesting to notice that this phenomenon was found only for the RAM results. This could be due to the higher sensitivity of the RAM to drug treatment possibly because of the prolonged food deprivation associated with this task. In both tasks, however, a repeated administration of AF102B at the same dose clearly improved the spatial cognitive performance of old rats during subchronic administration.

The first clear relationship to be established between age-related loss of memory and related cognitive functions and a dysfunction in neurotransmission involved the cholinergic system, known as the "cholinergic hypothesis" (4). The results of this study support the assertion of this hypothesis, namely that proper enhancement of cholinergic function may significantly reduce the severity of cognitive loss. These results may also provide a support for the involvement of cholinergic input in the observed impair-

ments of aging.

Whatever the positive results that have been claimed or obtained in previous studies with cholinergic agents, one must recognize that they were extremely subtle, quite variable and offered little or no significant therapeutic relief in daily living situations. Among the basic pharmacologic factors which were responsible for the lack of significant therapeutic effects were the pharmacokinetic properties of the available cholinergics tested to date, including extremely short half-lives, lack of specificity to the central nervous system (CNS), poor passage through the blood-brain barrier, high incidence of adverse side effects and extremely narrow therapeutic windows (12,27). Recent studies suggested the use of more specific muscarinic agonists as a treatment strategy for AD (25, 28, 31, 42, 50). Accordingly, M1 specific agonists may be more efficacious in improving overall cholinergic function and possibly reducing cognitive disturbances associated with old age, Alzheimer's disease and other clinical conditions characterized, in part, by a central cholinergic deficiency. AF102B was indeed shown to be devoid of side-effects up to high doses, resulting in a fairly wide therapeutic range (18). The development of this new drug with greater specificity and selectivity for a receptor subclass, M1, may provide a valuable tool for treating both AD, and age-related cognitive impairments, since it has been proved to have a unique activity in reversing learning and memory loss associated with aging.

ACKNOWLEDGEMENT

This work was supported by Snow Brand Milk Products Co. Ltd., Tokyo, Japan.

REFERENCES

1. Barnes, C. A. Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. 93:74–104; 1979.
2. Barnes, C. A. The physiology of the senescent hippocampus. In: Seifert, W., ed. Neurobiology of the hippocampus. London: Academic Press; 1983:87–108.
3. Barnes, C. A.; Nadel, L.; Honig, W. K. Spatial memory deficits in senescent rats. Can. J. Psychol. 34:29–39; 1980.
4. Bartus, R. T.; Dean, R. L., III; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
5. Bartus, R. T.; Flicker, C.; Dean, R. L. Logical principles for the development of animal models of age-related memory impairments. In: Crook, T.; Ferris, S.; Bartus, R., eds. Assessment in geriatric psychopharmacology. Madison: Mark Powley Associates; 1983:263–300.
6. Beatty, W. W.; Bierley, R. A. Scopolamine degrades spatial working memory but spaces spatial reference memory: Dissimilarity of anticholinergic effect and restriction of distal visual cues. Pharmacol. Biochem. Behav. 23:1–6; 1985.
7. Becker, J. T.; Walker, J. A.; Olton, D. S. Neuroanatomist basis of spatial memory. Brain Res. 200:307–320; 1980.
8. Buresova, O.; Bolhuis, J. J.; Bures, J. Differential effects of cholinergic blockade on performance of rats in the water tank navigation task and in a radial water maze. Behav. Neurosci. 100:476–482; 1986.
9. Clarke, D. J.; Gage, F. H.; Nilsson, O. G.; Bjorklund, A. Grafted septal neurons form cholinergic synaptic connections in the dentate gyrus of behaviorally impaired aged rats. J. Comp. Neurol. 252: 483–492; 1986.
10. Coyle, J. T.; Price, D. L.; Delong, M. R. Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
11. Davis, H. P.; Idowu, A.; Gibson, G. E. Improvement of 8-arm maze performance in aged Fischer 344 rats with 3,4-diaminopyridine. Exp. Aging Res. 9:211–214; 1983.
12. Davis, K. L.; Mohs, R. C.; Davis, B. M.; Rosenberg, G. S.; Horvath, T. H.; De Nigris, Y. Cholinomimetic agents in human memory: Preliminary observations in Alzheimer's disease. In: Pepen, G.; Ladinsky, H., eds. Cholinergic mechanisms: Phylogenetic aspects, central and peripheral synapses and clinical significance. New York: Plenum Press; 1981:929–936.
13. Decker, M. W.; Pelleymounter, M. A.; Gallagher, M. Effects of training on a spatial memory task on high affinity choline uptake in hippocampus and cortex in young adult and aged rats. J. Neurosci. 8:90–99; 1988.
14. De Toledo-Morrell, L.; Morrell, F.; Fleming, S. Age-dependent deficits in spatial memory are related to impaired hippocampal kindling. Behav. Neurosci. 98.902–907; 1984.
15. De Toledo-Morrell, L.; Geinisman, Y.; Morrell, F. Age-dependent alterations in hippocampal synaptic plasticity relation to memory disorders. Neurobiol. Aging 9:581–590; 1988.
16. Fisher, A.; Brandeis, R.; Karton, I.; Pittel, Z.; Sapir, M.; Grunfeld, Y.; Simon, G.; Rabinovitch, I.; Dachir, S.; Levy, A.; Heldman, E. *AF102B:* A potential drug for treatment of Alzheimer's Disease (AD): Further characterization. Soc. Neurosci. Abstr. 14:905; 1988.
17. Fisher, A.; Brandeis, R.; Pittel, Z.; Karton, I.; Sapir, M.; Dachir, S.; Levy, A.; Mizobe, F.; Heldman, E. *AF102B:* A new M1 agonist with potential application in Alzheimer's Disease (AD). Soc. Neurosci. Abstr. 13:657; 1987.
18. Fisher, A.; Brandeis, R.; Karton, I.; Pittel, Z.; Dachir, S.; Sapir, M.; Grunfeld, Y.; Levy, A.; Heldman, E. AF102B: A rationale treatment strategy in Alzheimer's disease (AD); recent advances In: Wurtman, R.; Corkin, S. H.; Growdon, J. H.; Ritter-Walker, E., eds. Alzheimer's Disease: Advances in basic research and therapies. Cambridge: Center for Brain Science and Metabolism Charitable Trust; 1989:703–707.
19. Fisher, A.; Heldman, E.; Brandeis, R.; Pittel, Z.; Dachir, S.; Levy A.; Karton, I. *AF102B:* A novel selective M1 agonist reverses AF64A-induced cognitive impairments in rats. Soc. Neurosci. Abstr. 12:702; 1986.
20. Fisher, A.; Heldman, E.; Brandeis, R.; Pittel, Z.; Dachir, S.; Levy, A.; Karton, I. Restoration of cholinergic and cognitive functions in an animal model of Alzheimer's Disease using various cholinergic manipulations. In: Dowdall, M. Y.; Hawthorne, J. N., eds. Cellular and molecular basis of cholinergic function. Chichester, Sussex: Ellis Harwood Series in Biomedicine; 1987:913–927.
21. Gage, F. H.; Bjorklund, A.; Stenevi, U. Intrahippocampal septal grafts ameliorate learning impairments in aged rats. Science 225: 533–536; 1984.
22. Gamzu, E. Animal behavioral models in the discovery of compounds to treat memory dysfunction. In: Olton, D. S.; Gamzu, E.; Corkin, S., eds. Memory dysfunctions: An integration of animal and human research from preclinical and clinical perspectives. New York: New York Academy of Science; 1985:370–393.
23. Gibson, G. E.; Peterson, C.; Jender, D. S. Brain acetylcholine synthesis declines with senescence. Science 213:674–676; 1981.
24. Hagan, J. J.; Salamone, J. D.; Simpson, J.; Iversen, S. D.; Morris, R. G. M. Place navigation in rats is impaired by lesions of medical septum and diagonal band but not nucleus basalis magnocellularis. Behav. Brain Res. 27:9–20; 1988.
25. Hagan, J. J.; Jansen, J. H. M. Broekkamp, C. L. E. Blockade of spatial learning by the M1 muscarinic antagonist pirenzepine. Psychopharmacology (Berlin) 93:470–476; 1987.
26. Hagan, J. J.; Tweedie, F.; Morris, R. G. M. Lack of task specificity and absence of post-training effects of atropine upon learning. Behav. Neurosci. 100:483–493; 1986.
27. Harbaugh, R. E.; Roberts, D. W.; Coombs, D. W.; Saunders, R. L.; Reeder, T. M. Preliminary report: Intracranial cholinergic drug infusion in patients with Alzheimer's disease. Neurosurgery 15: 514–518; 1984.
28. Hunter, A. J.; Roberts, F. F. The effect of pirenzepine on spatial learning in the Morris water maze. Pharmacol. Biochem. Behav. 30:519–523; 1988.
29. Kadar, T.; Silbermann, M.; Levy, A. Structural brain changes and working memory deficiency in aging rats. The biology of aging, Second International Serling Symposium: 57; 1988.

30. Levy, A.; Kluge, P. B.; Elsmore, T. F. Radial maze performance of mice: Acquisition and atropine effects. Behav. Neural Biol. 39: 229–240; 1983.
31. Mash, D. C.; Flynn, D. D.; Potter, L. T. Loss of M2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. Science 228:1115–1117; 1985.
32. Miyamoto, M.; Kato, J.; Narumi, S.; Nagaoka, A. Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Res. 419:19–31; 1987.
33. Mochida, S.; Mizobe, F.; Fisher, A.; Kawanishi, G.; Kobayashi, H. Dual synaptic effects of activating M1-muscarinic receptors in superior cervical ganglia of rabbits. Brain Res. 455:9–17; 1988.
34. Morris, R. G. M. Spatial localization does not require the presence of local cues. Learn. Motiv. 12:239–249; 1981.
35. Morris, R. G. M.; Garrud, P.; Rawlins, J. N. P.; O'Keefe, J. Place navigation impaired in rats with hippocampal lesions. Nature 297: 681–683; 1982.
36. Muller, W. E. Restoration of age-related receptor deficits in the central nervous system, a common mechanism of nootropic action. Methods Find. Exp. Clin. Pharmacol. 10:773–778; 1988.
37. Olton, D. S. Memory functions and the hippocampus. In: Seifert, W., ed. Neurobiology of the hippocampus. London: Academic Press; 1983:335–373.
38. Olton, D. S.; Becker, J. T.; Handelmann, G. E. Hippocampus, space and memory. Behav. Brain Sci. 2:313–322; 1979.
39. Olton, D. S.; Walker, J. A.; Gage, F. H. Hippocampal connections and spatial discrimination. Brain Res. 139:295–308; 1978.
40. Pearson, R. C. A.; Sofroniew, M. V.; Cuello, A. C.; Powell, T. P. S.; Eckenstein, F.; Esiri, M. M.; Wilcock, G. K. Persistence of cholinergic neurons in the basal nucelus in a brain with senile dementia of the Alzheimer's type demonstrated by immunohistochemical staining for choline acetyltransferase. Brain Res. 289:375–379; 1983.
41. Peele, O. B.; Baron, S. P. Effects of selection delays on radial maze performance: Acquisition and effects of scopolamine. Pharmacol. Biochem. Behav. 29:143–150; 1988.
42. Perry, E. K. The cholinergic hypothesis—ten years on. Med. Bull. 42:63–69; 1986.
43. Rapp, P. R.; Rosenberg, R. A.; Gallagher, M. An evaluation of spatial information processing in aged rats. Behav. Neurosci. 101: 3–13; 1987.
44. Smith, G. Animal models of Alzheimer's disease: Experimental cholinergic denervation. Brain Res. Rev. 13:103–118; 1988.
45. Sutherland, R. J.; Kolb, B.; Whishaw, I. Q. Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rats. Neurosci. Lett. 31:271–276; 1982.
46. Sutherland, R. J.; Whishaw, I. Q.; Regehr, J. C. Cholinergic receptor blockade impairs spatial localization by use of distal cues in the rat. J. Comp. Physiol. Psychol. 96:563–572; 1982.
47. Wallace, J. W.; Krauter, E. E.; Campbell, B. A. Animal models of declining memory in the aged: short-term and spatial memory in the aged rat. J. Gerontol. 35:353–363; 1980.
48. Weinberg, J. Geriatric psychiatry. In: Kaplan, H. I.; Freedman, A. M.; Sadock, B. J., eds. Comprehensive textbook of psychiatry. vol. 3. Baltimore: Williams & Wilkins; 1980:3024–3042.
49. Whishaw, J. Q.; O'Connor, W. T.; Dunnett, S. B. Disruption of central cholinergic systems in the rat by basal forebrain lesions or atropine: Effects of feeding, sensorimotor behavior, locomotor activity and spatial navigation. Behav. Brain Res. 17:103–115; 1985.
50. Whitehouse; P. J. Neuronal loss and neurotransmitter receptor alterations in Alzheimer's disease. In: Fisher, A.; Hanin, I.; Lachman, C., eds. Alzheimer's and Parkinson's Disease: Strategies for research and development. New York: Plenum Press; 1986:85–94.