Pharmacology Biochemistry and Behavior, Vol. 47, pp. 325-329, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Effect of AIT-082, a Purine Analog, on Working Memory in Normal and Aged Mice

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Received 22 March 1993

GLASKY, A. J., C. L. MELCHIOR, B. PIRZADEH, N. HEYDARI AND R. F. RITZMANN. *Effect of AIT-082, a purine analog, on working memory in normal and aged mice.* PHARMACOL BIOCHEM BEHAV 47(2) 325-329, 1994.—Because working memory is the primary type of memory which is disrupted by Alzheimer's disease and stroke and during aging, any therapeutic drug for these conditions should improve and/or restore working memory. The win-shift memory paradigm has been shown to be an excellent model of working memory. In the present study, we examined the effects of a novel purine derivative, 4-[[3-(1,6-dihydro-6-oxo-9-purin-9-yl)-1-oxopropyl]amino]benzoic acid (AIT-082) and physostigmine (PHY) on working memory. Both AIT-082 and PHY improved memory in young mice and restored memory in mice with mild age-induced memory deficits; however only AIT-082 was also effective in subjects with moderate deficits. Neither drug improved memory in mice with severe memory deficits. AIT-082 exhibited effectiveness over a broad dose range (0.5-60 mg/kg), and the effects lasted for seven days after a single high-dose drug administration. AIT-082 was devoid of any effects on performance variables and has not shown any toxic side effects, thus making it an interesting potential treatment for working memory deficits associated with aging, strokes, and Alzheimer's disease.

Working memory AIT-082 Mice Aging Memory deficit Purine Physostigmine Tacrine

LOSS of memory represents the core symptom of Alzheimer's disease. Since cholinergic drugs have had only limited success (4) in treating Alzheimer's dementia, it would be important to evaluate drugs with different mechanisms of action for potential therapeutic activity. A growing body of evidence has divided memory into several subtypes (i.e., working and reference memory) (10,14,16,18). Working memory is particularly disrupted in humans suffering from pathologies which produce dementias such as Alzheimer's disease and stroke. It has been proposed that working memory consists of the remembering of an event in the absence of specific sensory cues (11), in contrast to dispositional memory, where a set of specific cues are present and a choice between a correct and incorrect cue(s) is required.

Ordy et al. (11) have reported that, in rats, the win-shift paradigm specifically tests working memory. They have shown that this type of memory is disrupted by hippocampal lesions and ischemia and during aging in rats (11). We have shown (15) that mice perform well in this paradigm and that Swiss Webster mice develop memory deficits at approximately one year of age. By measuring the longest delay at which 11-month-old mice could perform above chance, we were able to group them into three categories: 1) mild deficit (30-s delay), 2) moderate deficit (10-s delay), and severe deficit (less than 10 s). Therefore, this model provides an excellent tool for testing drugs for use in treating these types of memory deficits.

In the present study, we tested a novel purine derivative for its ability to improve working memory in young mice as well as in mice with various degrees of age-induced memory deficit. 4-[[3-(1,6-Dihydro-6-oxo-9-purin-9-yl)-1-oxopropyl]-amino]benzoic acid (AIT-082), a derivative of the purine hypoxanthine, was synthesized based upon a report (6) that inosine pranobex, a mixture of inosine and a salt of *p*-acetamidobenzoic acid enhanced learning of a conditioned avoidance task in rats (5). Additionally, Rathbone (12) has proposed that extracellular purine nucleosides and nucleotides are primitive, ubiquitous intercellular messengers that, in higher animals, function as neuromodulators and local hormones. The purine adenosine is involved in the sequence of events from the activation of excitatory amino acid receptors to the establishment of long-term potentials (LTPs), presumably via nitric oxide (NO) (3). Since a number of purines mimic the actions of adenosine (12), it was reasoned that AIT-082 might increase activity in this system and therefore influence memory. Therefore, it was of interest to evaluate the effect of AIT-082

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TABLE 1

EFFECT OF DRUG TREATMENT ON WIN-SHIFT PERFORMANCE OF YOUNG SWISS WEBSTER MICE

	Control (Saline)	THA (1.25 mg/kg)	AIT-082 0.5 mg/kg	AIT-082 30.0 mg/kg	PHY (0.125 mg/kg)
Correct responses	4.6	7.1*	6.5*	8.2*	6.5*
Latency time	2.68	8.22*	1.95	2.03	7.82*
Running time	1.95	3.65*	2.20	1.95	2.65
Tolerance (Correct responses)	4.9	N/T	N/T	7.6*	N/T

Correct responses are the mean number of correct per 10 trials. $N = 20$ per group. Tolerance was measured after 18 days of daily administration of AIT-082 (30 mg/kg). *$p < 0.05$ (ANOVA).

on memory function. Tacrine (THA) and physostigmine (PHY), known cholinergic agents, were selected for comparison to AIT-082 because they have been shown to modify memory in rats (6,11).

METHODS

Animals

Male Swiss Webster mice 6 months of age (young adult) and 11 months of age (old) obtained from the National Institute on Aging were maintained in individual cages on a 12-h light/dark cycle with continuous access to water. Food was limited so that the mice stabilized at 80% of free feeding weight. Mice were weighed and handled daily for one week.

Drugs

The drugs used in this report are AIT-082, sodium salt (Advanced ImmunoTherapeutics, Tustin, CA); tacrine hydrochloride (tetrahydroaminoacridine [THA]) (Sigma Chemical Co., St. Louis); and physostigmine hemisulfate salt (PHY) (Sigma). The drugs were dissolved in saline and prepared fresh daily. All injections were made at a volume of 0.1 ml/10 g body weight. When testing drug effects, IP injections of AIT-082 or THA were made 1 h prior to the start of testing. Due to its shorter duration of action (17), PHY was injected 30 min prior to testing. Control subjects received a similar injection of saline (vehicle).

Win-Shift Memory Test

Subjects were shaped in a standard T-maze with the stem and each arm measuring 35 × 5 × 5 cm (L × W × H) by placing the subject in the maze for 3 min with all doors open and milk in both goal boxes. This process was repeated until the subject drank the milk. During tests of memory, the latency or time to leave the start box was recorded as an index of motivation. The time to traverse the maze (running time)

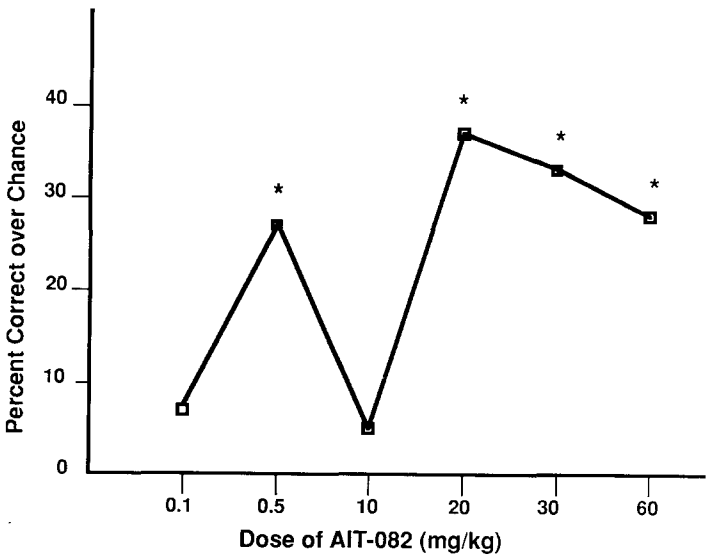

FIG. 1. Dose response of AIT-082 on working memory in young adult Swiss Webster mice. Values presented as mean score per group of 20 subjects. Data are expressed as percent correct responses over chance when tested at a 90-s intertrial interval. *$p < 0.05$ (t test).

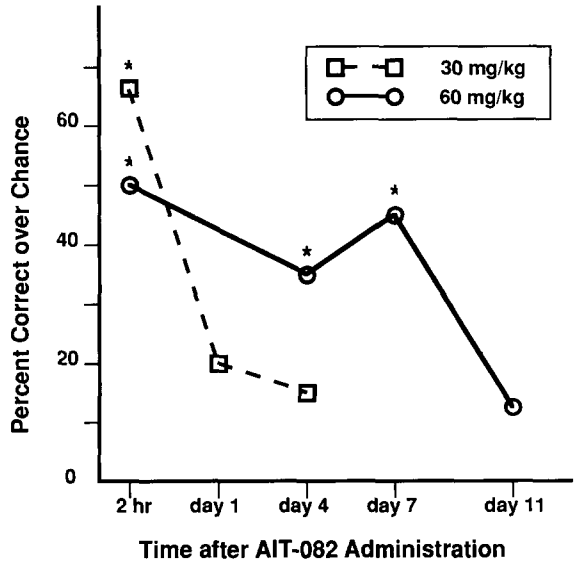

FIG. 2. Duration of action of AIT-082 on working memory in young adult Swiss Webster mice. Values presented as mean score per group of 20 subjects. Data are expressed as percent correct responses over chance when tested at a 90-s intertrial interval. *$p < 0.05$ (t test).

was recorded as a performance measure, and the arm of the maze entered was noted. At short intertrial delays (i.e., 30 or 60 s) mice with intact memory would enter the side of the maze opposite that which they had entered on the previous trial. Thus, entering the opposite side was designated as a correct response (win-shift). At the longer delays (i.e., 90 s), untreated mice performed at chance levels (i.e., they chose the opposite side only 50% of the time). The selection of the correct side resulted in the subjects receiving a reward of 0.5 cc of milk, which the subject was allowed to consume. During test trials, the reward was alternated after each correct response.

Experimental Design

To determine the duration of working memory, subjects were administered drug or saline and 30 min (PHY) or 1 h later (AIT-082 or THA) they were given a single reference run with the milk reward in both goal boxes. After the indicated intertrial delay, subjects were returned to the start box and given the first test trial with the milk reward only in the goal box opposite to the one entered on the previous correct trial. The subjects were given 10 trials with the reward alternating only after correct responses. The number of correct trials per set of 10 trials was recorded, as well as the latency time to leave the start box and the running time. The same procedure was repeated 24 to 72 h later, except the intertrial interval was increased. The schedule was repeated until the subject no longer responded above chance (i.e., greater than five correct responses per 10 trials).

Various doses of AIT-082 were administered to subjects tested at a 90-s intertrial interval to determine the dose response range. The duration of action of AIT-082 was determined by retesting subjects that had received 30 or 60 mg/kg of AIT-082 and who performed 100% correct on the first series of trials. These subjects were then retested at the indicated times post-drug treatment (i.e., at 2 h and 24 h and on the 4th, 7th, and 11th days after AIT-082 administration).

To test if tolerance developed to AIT-082, AIT-082 was injected at a dose of 30 mg/kg daily for 18 days. Control subjects received a similar regime of saline injections. Subjects were tested on the 1st and 18th days only.

Analyses of Data

Data are presented as the number of correct responses per 10 trials or as the percent correct responses over chance. Chance is defined as 50% correct responses. To determine if the performance was significantly above chance, a t test against a theoretical distribution was performed. Reciprocal transformations were performed on latency times to leave the start box, and arc sign transformations were performed on percentages, then analyzed by an analysis of variance (ANOVA) or t test. Significant F ratios were further analyzed by a Duncan's multiple range test.

RESULTS

Control young adult subjects tested in this model were able to perform significantly above chance (75% correct) at intertrial delays of 30 and 60 s. However, at delays of 90 s their

TABLE 2

EFFECT OF AIT-082 AND PHYSOSTIGMINE ON THE DURATION OF WORKING MEMORY IN MICE WITH AGE-INDUCED DEFICITS

Degree of Deficit	Intertrial Interval (s)	Control (Saline)	AIT-082 (30 mg/kg)	PHY (0.125 mg/kg)
Mild	60	0/6	6/6*	5/6*
	90		4/5*	3/5
	120		2/4	2/3
	150		1/2	2/2
	180		1/1	1/2
	210		0/1	0/1
Moderate	30	0/6	4/6*	1/6
	60		2/4	0/1
	90		0/2	
Severe	<10	0/6	0/6	0/6

Data are presented as the number of subjects performing significantly above chance/total number of subjects. *$p < 0.05$ (t test).

performance fell to a level not significantly different from chance ($p < 0.05$). AIT-082 (30 mg/kg), PHY (0.125 mg/kg), and THA (1.25 mg/kg) improved performance at the 90-s delay (Table 1). At the doses tested, AIT-082 did not have any effect on either latency time to leave the start box or the time to traverse the maze (running time). In contrast, PHY prolonged latency time and THA produced an increase in both latency and running time (Table 1).

Dose response and time course studies revealed that AIT-082 was active at doses from 0.5 to 60 mg/kg (Fig. 1). The effects of AIT-082 at a dose of 30 mg/kg were no longer apparent 24 h after drug administration; however, the effects of a dose of 60 mg/kg were still evident seven days after a single drug administration (Fig. 2).

Tolerance did not develop to AIT-082. When the drug was administered at a dose of 30 mg/kg daily for 18 days, there was no significant decrease in performance after 18 days of repeated drug treatment (Table 1).

In tests of memory in aged mice, 18 subjects were tested for duration of working memory and grouped into three categories: 1) those who could not perform at the 10-s delay (severe deficit), 2) those who could perform at a 10-s delay but not at 30 s (moderate deficit), and 3) those who could perform at 30 s but not at 60 s (mild deficit).

The results of testing AIT-082 and PHY in the three groups of aged animals is presented in Table 2. AIT-082 (30 mg/kg) improved performance significantly ($p < 0.05$) in mice with mild memory deficits by prolonging the duration of working memory from 30 s to 90 s and in the moderate memory deficit group from less than 10 s to 30 s. PHY produced significant improvement in the mild deficit group but not in the moderate deficit group. Neither drug produced any effect in the severe deficit animals.

DISCUSSION

These results confirm our earlier observations (15) that young adult mice perform well in the win-shift memory testing paradigm and that this model can detect varying degrees of memory deficit in aged Swiss Webster mice. This paradigm provides a model to compare the efficacy of drugs in age-matched subjects with varying degrees of working memory impairment.

In normal young adult Swiss Webster mice, AIT-082 prolonged the duration of working memory. Dose response and time course studies indicated that AIT-082 was active over a wide range of doses (0.5–60 mg/kg) and at the higher dose (60 mg/kg) the effects lasted for up to seven days. PHY, an anticholinesterase agent, has been demonstrated to have memory-enhancing activity in animals (1,11) and has been shown to be effective in the win-shift paradigm in rats (11). THA has memory-enhancing activity (6) and may possibly act through a cholinergic mechanism (17). Both PHY and THA prolonged the duration of working memory in young adult mice in this paradigm. However, only with AIT-082 was there an absence of any other effect on performance (i.e., latency time to leave the start box or the running time). While typically in this type of paradigm prolonged latency time is reflective of motivation and prolonged running time is reflective of motor effects (10), it is unclear whether the effects of PHY and tacrine on these parameters are due to these factors or to other toxic side effects reported for these compounds.

In tests of working memory in aged matched 11-month-old mice with varying degrees of impairment, AIT-082 was found to increase the duration of the working memory longer than PHY in both the mild and moderate deficit groups. Neither drug treatment produced any improvement in the severe deficit group.

Purines modulate a number of cellular functions, including proliferation of astrocytes (13) and immune cells (2) as well as neurite outgrowth in PC12 cells in tissue culture (12). The purines guanosine, inosine, and hypoxanthine have been shown to stimulate neurite outgrowth in PC12 cells (12); however, since they are poor agonists for the adenosine receptors (19), they probably act through a different receptor (8). Preliminary results (Rathbone, personal communication) have demonstrated that AIT-082 stimulates neurite outgrowth in PC12 cells and enhances the effect of nerve growth factor (NGF) in that system. NGF has been reported to increase cyclic GMP in PC12 cells (7). NO has been proposed as the mediator of LTP, a form of memory, and it has been suggested that NO's activity may be through stimulation of the production of cGMP (9). These observations raise the possibility that AIT-082 may exert its activity through a cGMP-dependent mechanism.

Since cholinergic drugs like THA and PHY have had only limited success in treating Alzheimer's dementia (4), it is important to have additional potential therapeutic agents that enhance and restore working memory while acting through a different mechanism. The data from the present experiments indicate that AIT-082, a purine analogue, improves memory in an intact animal and reverses age-induced memory deficits. AIT-082 appears to be an excellent candidate for therapeutic consideration due to its broad dose response range, its long duration of action, and the absence of toxicity in animals. Additional studies will be required to elucidate the molecular site of action of AIT-082, with particular emphasis being directed to modulation of second messenger systems.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Institutes of Health (AG09911 and AA08709) and the Veterans Administration Research Service.

REFERENCES

1. Bartus, R. T. Drugs to treat age-related neurodegenerative problems: The final frontier of medical science? J. Am. Geriatr. Soc. 38:680–695; 1990.
2. Coffey, R. G.; Hadden, J. W. Calcium and guanylate cyclase in lymphocyte activation. In: Hadden, J. W.; Chedid, L.; Dukor, P.; Spreafico, F.; Willoughby, D., eds. Advances in immunopharmacology, vol. 2. Oxford, UK: Pergamon Press; 1983:87–94.
3. Collingridge, G. L.; Harvey, J.; Frenguellli, B. G.; Bortolotto, Z. A.; Bashir, Z. I.; Davies, C. H. Amino acid receptors and long-term potentiation: Targets for the development of cognitive enhancers. In: Racagni, G.; Mendlewicz, J., eds. Treatment of age-related cognitive dysfunction: Pharmacological and clinical evaluations. Basel, Switzerland: Karger; 1992:41–49.
4. Davis, K. L.; Thal, L. J.; Gamzu, E. K. A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. N. Engl. J. Med. 327:1253–1259; 1992.
5. Doty, B.; Gordon, P. Inosine-alkylamino alcohol complexes: Enhancement of conditioned avoidance learning in rats. Fed. Proc. 29:684; 1970.
6. Flood, J. F. Effect of acute arecoline, tacrine and arecoline +

tacrine posttraining administration on retention in late middle-aged mice. J. Gerontol. 43:854–856; 1988.
7. Laasberg, T.; Pihluk, A.; Neuman, T.; Paves, H.; Saarma, M. Nerve growth factor increases the cyclic GMP level and activates the cyclic GMP phosphodiesterase in PC12 cells. FEBS Lett. 239: 367–370; 1988.
8. Lorenzen, A.; Fein, T.; Schwabe, U. Characterization and purification of a novel high affinity adenosine binding protein from bovine brain membranes. Int. J. Purine Pyrimidine Res. 3:18; 1992.
9. O'Dell, T. J.; Hawkins, R. D.; Kandel, E. R.; Arancio, O. Tests of the roles of two diffusible substances in long-term potentiation: Evidence for nitric oxide as a possible early retrograde messenger. Proc. Natl. Acad. Sci. U. S. A. 88:11285–11289; 1991.
10. Olton, D. S. Strategies for the development of animal models of human memory impairments. 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 Sciences; 1985:113–121.
11. Ordy, J. M.; Thomas, G. J.; Volpe, B. T.; Dunlap, W. P.; Colombo, P. M. An animal model of human-type memory loss based on aging, lesion, forebrain ischemia and drug studies with the rat. Neurobiol. Aging 9:667–683; 1988.
12. Rathbone, M. P.; Christjanson, L.; DeForge, S.; Deluca, B.; Gybers, J. W.; Hindley, S.; Jovetich, M.; Middlemiss, P.; Takhal, S. Extracellular purine nucleosides stimulate cell division and morphogenesis: Pathological and physiological implications. Med. Hypotheses 37:232–240; 1992.
13. Rathbone, M. P.; Middlemiss, P. J.; Gysbers, J. W.; DeForge, S.; Costello, P.; DelMaestro, R. F. Purine nucleosides and nucleotides stimulate proliferation of a wide range of cell types. In Vitro Cell. Dev. Biol. 28A:529–536; 1992.
14. Rawlins, J. N. P. Associations across time: The hippocampus as a temporary memory store. Behav. Brain Sci. 8:479–496; 1985.
15. Ritzmann, R. F.; Kling, A.; Melchior, C. L.; Glasky, A. J. Effect of age and strain on working memory in mice as measured by win-shift paradigm. Pharmacol. Biochem. Behav. 44:805–807; 1993.
16. Sherry, D. F.; Schachter, D. L. The evolution of multiple memory systems. Psychol. Rev. 94:439–454; 1987.
17. Tachiki, K. H.; Spidel, K.; Samules, L.; Ritzmann, R. F.; Steinberg, A.; Lloyd, L.; Summers, W. K.; Kling, A. Tacrine: Levels and effect on biogenic amines and their metabolites in specific areas of the rat brain. In: Giacobini, G.; Becker, D., eds. Current research in Alzheimer's therapy. New York: Guilford Press; 1988: 217–220.
18. Thomas, G. T.; Gash, D. M. Differential effects of posterior septal lesions on dispositional and representational memory in the rat. Behav. Neurosci. 100:712–719; 1986.
19. Trivedi, B. K.; Bridges, A. J.; Bruns, R. F. Structure-activity relationships of adenosine-A1 and A2 receptors. In: Williams, M., ed. Adenosine and adenosine receptors. Clifton, NJ: Humana Press; 1990:57–104.