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Paeoniflorin Attenuates Learning Impairment of Aged Rats in Operant Brightness Discrimination Task

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OHTA, H., K. MATSUMOTO, M. SHIMIZU AND H. WATANABE. *Paeoniflorin attenuates learning impairment of aged rats in operant brightness discrimination task.* PHARMACOL BIOCHEM BEHAV **49**(1) 213–217, 1994. – The effects of paeoniflorin isolated from peony were examined on an aging-induced learning deficit in an operant brightness discrimination task in Fischer 344 rats. Learning in aged (25 months) rats was significantly impaired compared with young (5 months) rats. Daily administration of paeoniflorin (0.01 mg/kg, PO) significantly attenuated the learning impairment in aged rats, whereas it did not affect the learning in young rats. Although tacrine (0.3 and 1 mg/kg, IP), a cholinesterase inhibitor, also did not affect the learning in young rats, it slightly augmented the aging-induced learning deficit in the present task. These data indicate the therapeutic potential of paeoniflorin in the treatment of senile dementia and aging-induced cognitive dysfunction.

Discrimination learning Paeoniflorin Tacrine Aging

PAEONIFLORIN is a major constituent of peony root, a herb that has long been used in traditional Chinese herbal prescriptions to treat certain types of dementia. Recently, paeoniflorin as well as aqueous extract of peony root has been demonstrated to attenuate scopolamine-induced deficit in radial maze performance (13–15). These findings strongly suggest that paeoniflorin has beneficial effects on memory impairment in rats. However, to clarify its therapeutic potential in the treatment of senile dementia, it is necessary to examine the effects of paeoniflorin on other cognitive dysfunction models, such as aged animals.

Memory impairments in aged animals have been reported using a variety of behavioral tasks (1,3,5,6,12). Because the central cholinergic functions are well known to be deteriorated in aged animals (10,11), it is believed that this dysfunction may be the cause of age-related memory deficits. However, cholinomimetic pharmacotherapy for aging-induced memory deficits has met with only limited success (2). In aged rats, other neurotransmitter systems, especially the noradrenergic system, have also been shown to be impaired. Decreases in both norepinephrine content and turnover were observed in the brain of aged rats (17). Adrenoceptor binding was also reported to decrease with age (9). Recent evidence has suggested a contribution of noradrenergic dysfunction to the age-related memory deficits (7,8,21). These facts raise the possibility that pharmacological manipulation of noradrenergic function in aged rats may affect aging-induced memory deficits.

In a previous report (16), we demonstrated that aged Fischer rats exhibit impairment of learning but not retention in a simple operant discrimination task. Paeoniflorin has already been suggested to attenuate memory deficits following cholinergic dysfunction via adrenergic mechanisms (14,15). In the present study, the effects of paeoniflorin on an aging-induced deficit in the discrimination learning were examined in Fischer

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rats, and the effects were compared with those of 9-amino-1,2,3,4-tetrahydroacridine (tacrine), a potent cholinesterase inhibitor.

METHOD

Animals

Young and aged male Fischer 344 rats, 5 and 25 months of age at the beginning of the experiments, respectively, were used. The young and aged rats were purchased from Charles River Japan (Atsugi, Japan) at the age of 2 and 18 month, respectively, and housed in our SPF laboratory colony until the experiments. Three or four animals were housed in a cage and allowed free access to water. The temperature (25 ± 1°C) and humidity (60 ± 10%) were controlled under a 12 L : 12 D cycle (lights on, 0730 h). Aged and young animals were maintained on a mild food deprivation schedule to keep their body weight at approximately 80% and 85% of their free-feeding levels, respectively. Prior to these experiments, they were handled for 5–10 min per day for 3 days.

Apparatus

The rats were trained in a standard rodent Skinner cage (31 × 29 × 23 cm, MATYS, Japan) equipped with two retractable levers placed 15 cm apart and 5 cm above the grid floor. In the present experiments, only the right lever was used. Pressing the lever was reinforced by a food pellet (45 mg, Bio-Serv, USA). A food pellet receptacle was mounted 3 cm above the floor at an equal distance between the levers. The test cage was housed in a sound-attenuating cubicle equipped with a 6 W house light that was used as a cue signal. A dim light located above the lever was always on during each experimental session. Stimulus application, food supply, and recording of response number were controlled using a microcomputer.

Procedure

The behavioral procedure was detailed in our previous report (16). Briefly, the animals were first adapted to the test cage and trained to press the lever for food reinforcement (FR1). This schedule was progressively increased in ratio until an FR10 schedule was attained. When the number of response stabilized (more than 20 pressings/min), the houselight was alternately turned on and off in 3-min blocks for one 24-min session. This pretest session permitted an assessment of neophobic reaction to lights off. After the pretest session, brightness discrimination training began. Each training session consisted of FR10 reinforcement for lever presses during periods when the house light was on (S+) and of no reinforcement for presses during periods when the light was off (S−). The duration of a S+ or S− period was 3 min and each period was presented alternately. The total duration of a session was 24 min. The percentage of incorrect responses, the number of responses during S− period divided by the number of total responses, was calculated.

Drug Tests

Paeoniflorin was isolated and purified from peony root (*Paeonia lactiflora* PALLAS) as described by Shibata et al. (19). Either paeoniflorin or water was administered PO 90 min before daily training. When testing tacrine (Sigma, St. Louis, MO), either saline or tacrine was injected IP 30 min before daily training. Paeoniflorin and tacrine were dissolved in water (pH ~ 7.0) and saline, respectively, just before the experiments, and administered in a volume of 0.2 and 0.1 ml/100 g body weight, respectively.

Statistics

Differences between the percentages of incorrect responses throughout training sessions were analyzed using two-way analysis of variance (ANOVA).

RESULTS

Discrimination Learning in Aged Rats

The rats of the both young and aged groups were shaped to comparable baseline performance levels. In the pretest session, the number of responses during the S− period was about 50% of the total responses in both groups, suggesting no neophobic reactions to the discriminative stimulus (darkness) (Fig. 1, upper panel). Performance of aged rats in the first training session did not differ from that of young rats. Aged

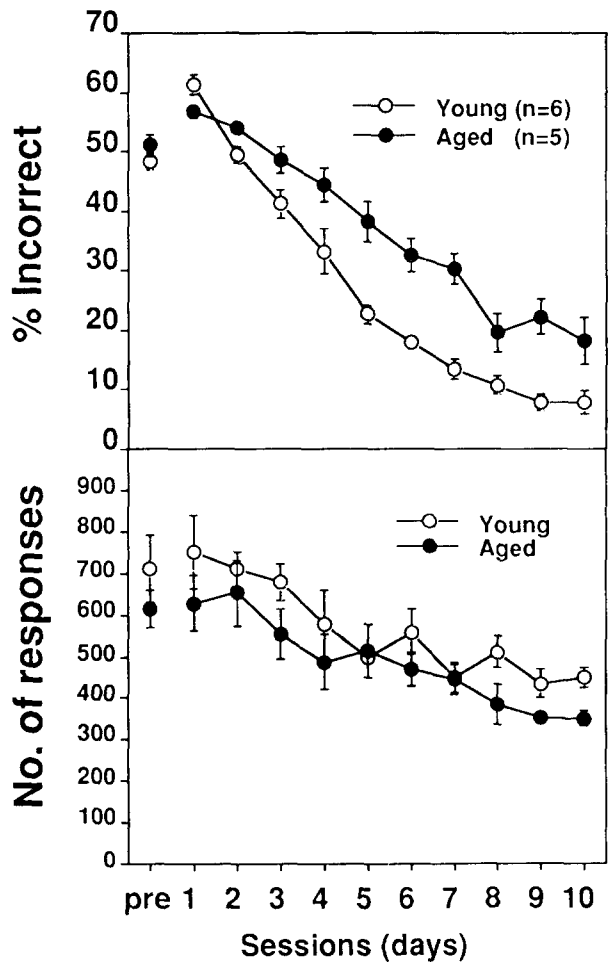

FIG. 1. Learning curves of brightness discrimination performance in young and aged Fischer rats. Rats were trained to discriminate brightness for food reward. Percentage of incorrect responses (upper panel) represents the number of responses during nonreinforced periods divided by the total number of responses (lower panel). The percentage 50 indicates no discrimination.

rats, however, showed significantly impaired learning compared with young rats in subsequent training sessions (Fig. 1, upper panel). A two-way ANOVA revealed a significant group difference, $F(1, 90) = 66.1$, $p < 0.001$, and a significant group × sessions interaction, $F(9, 90) = 2.8$, $p < 0.01$. The final levels of the percentage of incorrect responses were about 8% and 20% for young and aged groups, respectively. No marked difference was observed in terms of the number of total responses between young and aged rats throughout the training sessions (Fig. 1, lower panel).

Effects of Paeoniflorin on Discrimination Learning

Daily administration of paeoniflorin (0.01 and 0.1 mg/kg) neither affected the brightness discrimination learning nor changed the number of responses in young rats (Fig. 2). A two-way ANOVA indicated no group difference among the three groups in terms of either the percentage of incorrect responses or the number of responses. On the other hand, daily treatment with paeoniflorin (0.01 mg/kg) attenuated the discrimination learning in aged rats (Fig. 2). A two-way ANOVA revealed a significant difference between aged rats who received paeoniflorin and who did not, $F(1, 174) = 16.1$, $p < 0.001$. This treatment did not affect the number of responses in the aged rats (Fig. 2). Although we tested the effect of paeoniflorin at a higher dose (0.1 mg/kg) using the lesser number of aged rats ($n = 4$), the discrimination learning in aged rats with the paeoniflorin treatment was not statistically different from that in aged control rats.

Effects of Tacrine on Discrimination Learning

Daily injection of tacrine (1 mg/kg) did not affect either the discrimination learning or the number of responses in young rats (Fig. 3). No significant differences were observed between two groups in terms of either the percentage of incorrect responses or the number of responses. In aged rats, however, tacrine slightly but significantly augmented the learning impairment (Fig. 3) at 0.3 and 1 mg/kg, $F(1, 127) = 15.3$, $p < 0.001$; $F(1, 103) = 15.3$, $p < 0.001$, respectively. Tacrine did not change the number of responses (Fig. 3).

DISCUSSION

In agreement with our previous study (16), aged Fischer rats showed impaired learning of the brightness discrimination performance compared to young rats. The fact that the number of total responses in aged rats was not different from that in young rats throughout the training sessions suggests that there was no motor dysfunction in the aged rats and that the two age groups were equally motivated. Thus, the learning impairment observed in aged rats is considered to be due to a deficit in the acquisition of reference memory.

Daily administration of paeoniflorin significantly attenu-

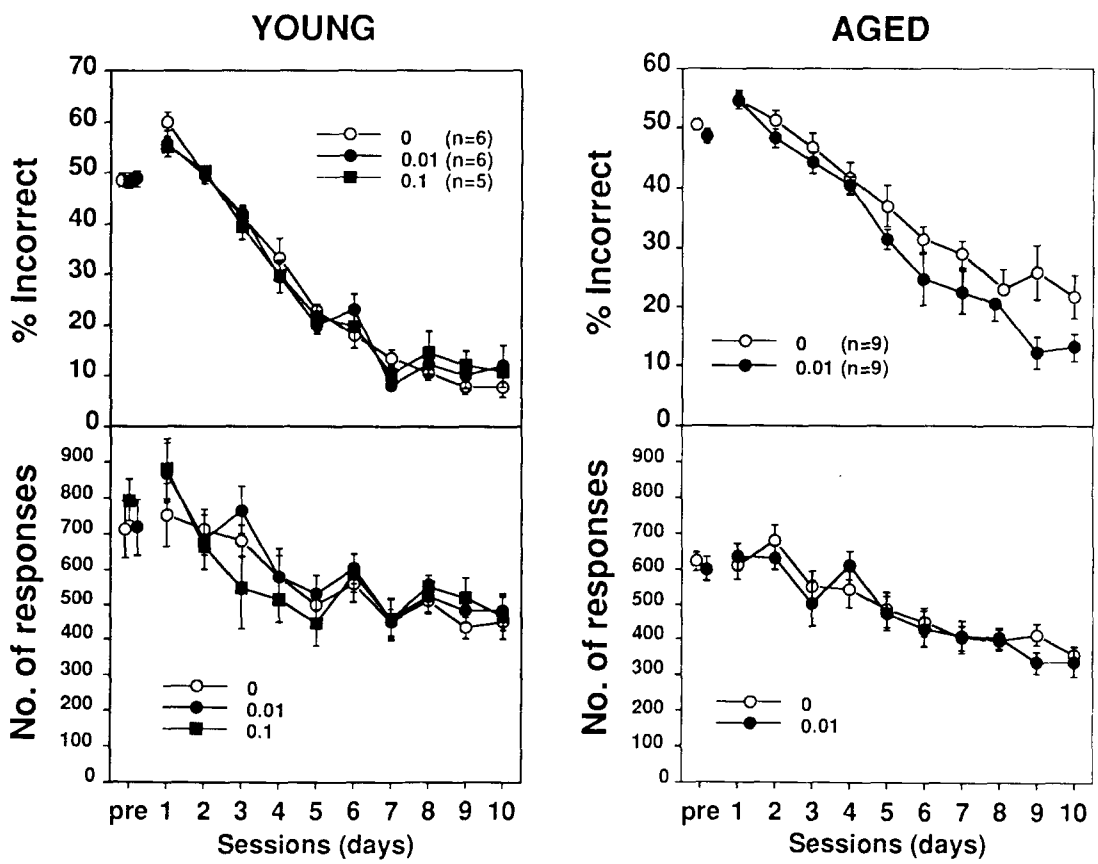

FIG. 2. Effects of paeoniflorin on brightness discrimination learning in young (left panel) and aged (right panel) rats. Other explanations are as in Fig. 1. Paeoniflorin (0.01 or 0.1 mg/kg) was orally administered 90 min before daily training sessions.

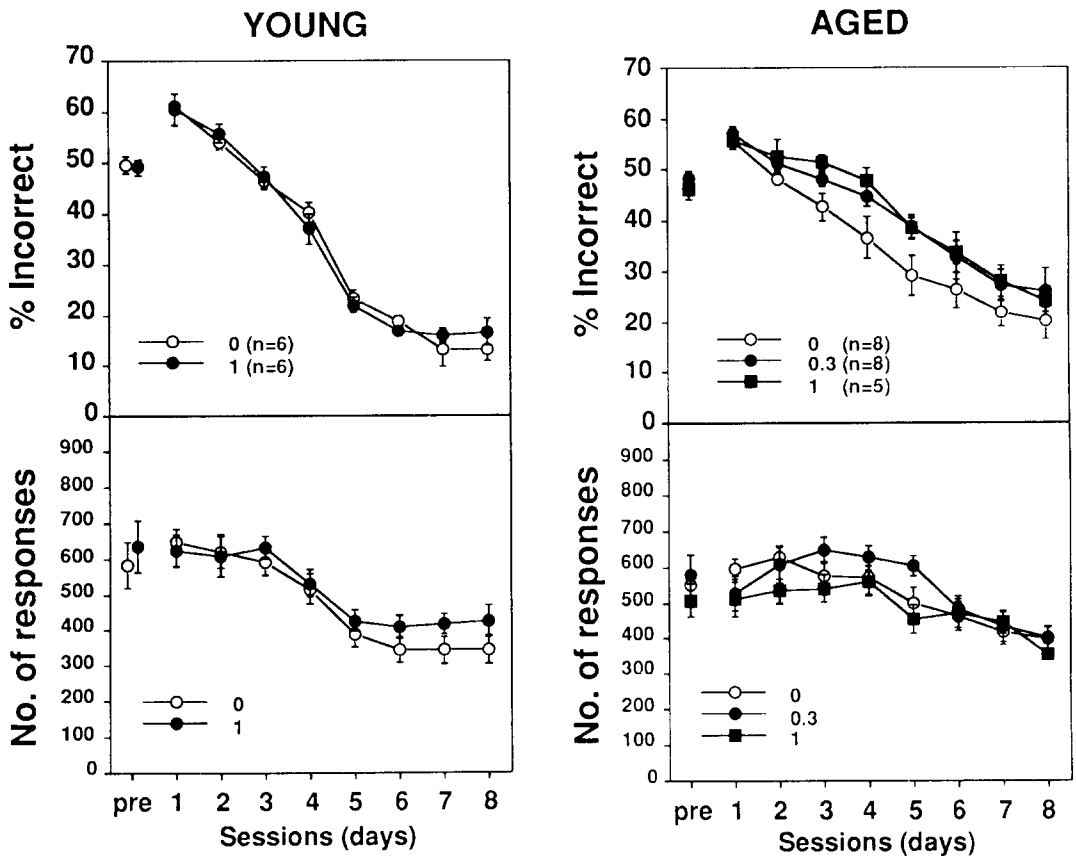

FIG. 3. Effects of tacrine on brightness discrimination learning in young (left panel) and aged (right panel) rats. Other explanations are as in Fig. 1. Tacrine (0.3 or 1 mg/kg) was intraperitoneally administered 30 min before daily training sessions.

ated the impaired learning in aged rats without affecting the learning in young rats. This agent has been shown to attenuate scopolamine-induced memory deficit in an eight-arm radial maze task (13-15). In addition, a recent experiment in our laboratory has shown that paeoniflorin improves the spatial learning deficits produced by unilateral nuleus basalis magnocellularis (nBM) lesion (unpublished data). These findings strongly suggest the possible utility of paeoniflorin in the treatment of dementia and age-related loss of cognitive function. In the present study, the administration of paeoniflorin over 10 days did not produce any observable adverse effects in aged rats or in young rats. This is consistent with a previous report demonstrating the extremely low toxicity of this agent (20). Because paeoniflorin has not been shown to produce any other pharmacological effect at the doses tested in the present study (20), the effects of this agent on cognitive functions seem to be highly specific. These facts indicate a potential advantage of the use of paeoniflorin in the treatment of senile dementia.

In contrast to paeoniflorin, tacrine did not improve, but even slightly augmented, the learning impairment in aged rats. One possible explanation for the result might be the lack of optimal tacrine doses in the present study, because the dose range within which tacrine has been shown to produce cognitive-enhancing effects is narrow (18). The other explanation is that the age-related learning impairments in the present study may not be due to dysfunction of the cholinergic system alone. Riekkinen et al.(18) have demonstrated that although tacrine improves memory deficits following nBM lesions, the beneficial effect on nBM-lesion-induced memory deficits is blocked by frontal cortex lesions. This suggests that tacrine does not improve memory deficits produced by widespread brain damage including noncholinergic neuronal systems. Clinical data also have demonstrated the lack of improvement by cholinesterase inhibitors in the treatment of geriatric memory disorders (4).

In our previous report (16), daily pretrial injections of scopolamine failed to impair the learning of the same discrimination performance as in the present study, even at the dose that markedly suppressed the number of responses in young rats. This finding may support the hypothesis that the aging-induced impairment observed in the present study is not only due to the age-related deficit in the cholinergic function. In other preliminary experiments, we have found that concurrent treatment with scopolamine and propranolol markedly impairs the present discrimination learning in young rats, although both antagonists by themselves produce no effect. These findings suggest that the age-related learning deficit in the present discrimination task may be due to concurrent impairments of both β-adrenergic and cholinergic functions. In our previous report (15), the enhancing effect of paeoniflorin on scopolamine-induced radial maze performance deficit was

completely blocked by β-blockers at doses that neither impaired the maze performance by themselves nor affected the scopolamine effect. These results suggest that paeoniflorin produces its cognitive-enhancing effects, at least in part, via the β-adrenergic system. Thus, it is likely that the effect of paeoniflorin on age-related learning deficit is attributable to activation of the β-adrenergic system. Further studies may be needed to elucidate the mechanism underlying the paeoniflorin effect on aging-induced memory deficits.

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