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BRIEF COMMUNICATION

Effects of Medetomidine, a Selective α_2-Agonist, on Position Discrimination and Reversal Learning in Aged Rats

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TANILA, H. *Effects of medetomidine, a selective α_2-agonist, on position discrimination and reversal learning in aged rats.* PHARMACOL BIOCHEM BEHAV **44**(2) 475–480, 1993.—The effect of a low dose (3 μg/kg) of medetomidine on cognitive performance of aged rats was examined in position discrimination and reversal learning in a T-maze. In Experiment 1, the reversal was introduced after 3 days of position habit acquisition and in Experiment 2 in the same session. Open-field and novelty-suppressed feeding tests were undertaken before drug testing to relate the cognitive effect of medetomidine with exploratory activity and neophobia of individual rats. Medetomidine slightly impaired the acquisition of position discrimination in both sexes and decreased perseveration in the reversal of a well-established position habit in females. The correlation of these findings with exploratory activity and neophobia is discussed.

Aging Rat Noradrenaline α_2-adrenoceptor Position discrimination Spatial reversal Open field Novelty-suppressed feeding

AGE-related degeneration of the noradrenergic system of the brain has been documented in rodents (15,16,21), monkeys (6,12), and humans (27,31). α_2-Adrenergic agonists have been shown to alleviate the poor performance of aged monkeys in delayed response (1) and delayed nonmatch-to-sample tasks (2) but not to affect visual discrimination performance (1). In a recent longitudinal study in our laboratory, a low dose of medetomidine improved delayed alteration performance of aged but not young, male rats (9). On the other hand, low doses of guanfacine have been reported not to improve spatial memory of old, female rats in a water maze (23).

Which cognitive function in aged animals is enhanced by α_2-agonists is an issue far from settled. Compared with young individuals, aged animals show rapid forgetting (5,18), sensitivity to distraction (3), behavior rigidity and perseverative tendency (4,18), increased neophobia (24), and retarded habituation to novel environment (17). All these deficits may have contributed to the impaired performance of old animals in delayed tasks. Further, depletion of brain noradrenaline has been reported to result in similar deficits (8,19,20,25,26).

Medetomidine is a highly selective and potent α_2-agonist with α_2/α_1 binding selectivity ratio of 1620 compared with 220 of clonidine (28). In the previous study from our laboratory, the most significant improvement in the cognitive performance of aged rats was obtained by medetomidine 3 μg/kg IM, about one tenth of the smallest sedative dose (22). Thus, this dose was chosen to study the effects of medetomidine on aged rats in position discrimination and reversal tasks. It was hypothesized that if the treatment reduces forgetting or sensitivity to distraction it should equally improve the acquisition of position discrimination and reversal, whereas only reversal learning should be enhanced if the perseverative tendency is alleviated. Further, to evaluate effects on neophobia and explorative activity as causes of the cognitive enhancement rats were also tested in novelty-suppressed feeding and open-field tasks.

METHOD

Animals

Naive Wistar rats (National Animal Center, Kuopio, Finland), 11 males (weighing 490–635 g) and 13 females (285–505 g), were used in the present study. At the beginning of the testing, rats were 27 (one rat) and 29–31 months old. Rats were housed singly in mounted rack cages (temperature 21–24°C, humidity 45–55%, light on from 0600–1800 h). Controlled rations of standard food pellets were delivered after a daily training or testing session; water was given ad lib. Two

males and two females died during the training. One female rat did not start eating rewards after extensive training and was excluded. Thus, the experiments were run with 9 males and 10 females. Two rats died soon after the experiment but did not show overt signs of illness during the test. The same rats were used in both experiments of the present study.

Apparatus

Testing was conducted in a quiet room with an illumination of 280 lux. Two identical T-mazes, one for males and the other for females, were used. The mazes were placed on a table, 1 m high. The T-maze was constructed of plywood, and was painted greyish-green. It consisted of a stem (76 × 15 × 15 cm), and two arms (57 × 15 × 15 cm), including a food recess. The starting compartment and arms could be closed by guillotine doors. The open field was a circular arena (83 cm in diameter) covered with lacquered cork. The walls of the arena, 50 cm high, were painted white. The floor had three concentric black circles, which were subdivided by radial black lines into 19 segments of equal area. To prevent the influence of odor cues, both the T-maze and open field were wiped with a wet cloth after each trial.

Pretesting and Training

At the start of the study, the exploratory activity of rats was determined with a 2-min open-field test. The number of ambulations (floor segments entered with all four feet) and rearings on hind feet were counted. A food restriction was introduced to slowly reduce weights of rats to 85% of the free-feeding weight. Rats were handled and weighed daily. In the next phase, rats were familiarized with the T-maze by letting them explore it for 15 min daily until they repeatedly ate rewards at both ends of the maze. The number of sessions needed for rats to start eating in the maze was taken as a measure of their neophobia. In the third phase of training, a session of 10 trials was given twice a week. A piece of food pellet (c. 100 mg for males, c. 80 mg for females) or a piece of peanut of similar size was placed in the food recess of both arms. (Most rats preferred peanuts to standard pellets, and some could not eat hard pellets because of poor teeth.) The rat was allowed a free choice between the two arms, but after entering the arm it was prevented from returning by a guillotine door. Two consecutive runs to the same arm led to a forced choice to the opposite arm on the next trial. Between trials, the rat was lifted into a round bucket under the table for 3 min. The training was finished when rats left the starting compartment within 10 s, ate the rewards on each trial, and did not show clear position habit. This took 6–13 sessions (mean 8.8 sessions).

Experiment 1: Position Discrimination Learning and Reversal in Separate Sessions

Both experiments were run under double-blind conditions. Rats were divided into two groups matched according to sex, open-field activity, and neophobia in the maze. The test group received medetomidine (Domitor 1 mg/ml, Farmos Group Ltd., Turku, Finland) 3 μg/kg IM and controls an equal volume of saline 15 min before each daily session. After injections, rats were moved in their cages to the testing room. Rats were tested in squads of four using two identical T-mazes so that two rats were given trials alternatingly in the same maze. The floor of the maze was wiped with a wet cloth after each trial to prevent olfactory cues. The initial trial was a test run without reward to show the initial position preference of the rat. On the next trial, the rat was rewarded for entering the arm opposite the arm of the first choice. This arm remained the baited one. When the rat entered the goal arm, it was confined there for 30 s. An intertrial interval (ITI) of 3 min separated trials. The test was carried out until the criteria of 10 correct choices and 5 consecutive correct choices were met or when 30 trials had been run. The test was repeated on 2 following days with the reward in the same arm as in the first session. On the fourth day, the side of the reward was changed. The mean number of errors to the criterion in each session was calculated by summing the errors to 10 correct choices and 5 consecutive choices and dividing the sum by 2. The number of consecutive erroneous choices in the beginning of the reversal session was taken as a measure of perseveration.

Experiment 2: Place Discrimination Learning and Reversal in the Same Session

All rats had two test sessions separated by 2 or 3 days. On one day, they received medetomidine 3 μg/kg IM and on the other day saline. The order was counterbalanced. The injection and test procedures were similar to those in Experiment 1, and trials were separated by a 3-min ITI as in the previous experiment. On the first run, the rat had a free choice of either arm and was rewarded. Choosing the same arm repeatedly was rewarded until seven correct choices were made. Then, the side of the reward was changed and the test was carried out until the rat had chosen the new side 7 times or 30 trials had been run. The number of errors to reach the criterion of seven correct choices was calculated separately both for acquisition and reversal. The number of consecutive erroneous choices after reversal was taken as a measure of perseveration.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to compare the effects of sex, treatment, and training session on the mean number of errors to the criterion (repeated measures) and also to compare the sexes in open-field and neophobia tests. The Mann–Whitney *U*-test was used to determine the effect of treatment on perseverations. The correlation between behavioral parameters (exploratory activity and neophobia) and T-maze performance for each individual animal was determined using Spearman rank correlations.

RESULTS

Females were more active in the open field than males [for ambulations, $F(1, 15) = 20.7$, $p < 0.001$, and for rearings, $F(1, 15) = 8.2$, $p = 0.01$]. Both sexes needed 4 days on average (variation 1–7 days) to start eating in the maze. No significant sex effect was found in the acquisition of position discriminations, but in the reversal of Experiment 1 females made more errors (13.4 ± 2.1 vs. 6.6 ± 1.3, mean ± SEM) than males, $F(1, 15) = 8.4$, $p = 0.01$. However, the reversal in the same session (Experiment 2) was mastered equally by the sexes.

The three sessions in Experiment 1 seem to be enough for rats to acquire a position habit. The repeated-measures ANOVA showed a highly significant session main effect on acquisition, $F(2, 30) = 11.0$, $p < 0.001$ (Fig. 1), but the dif-

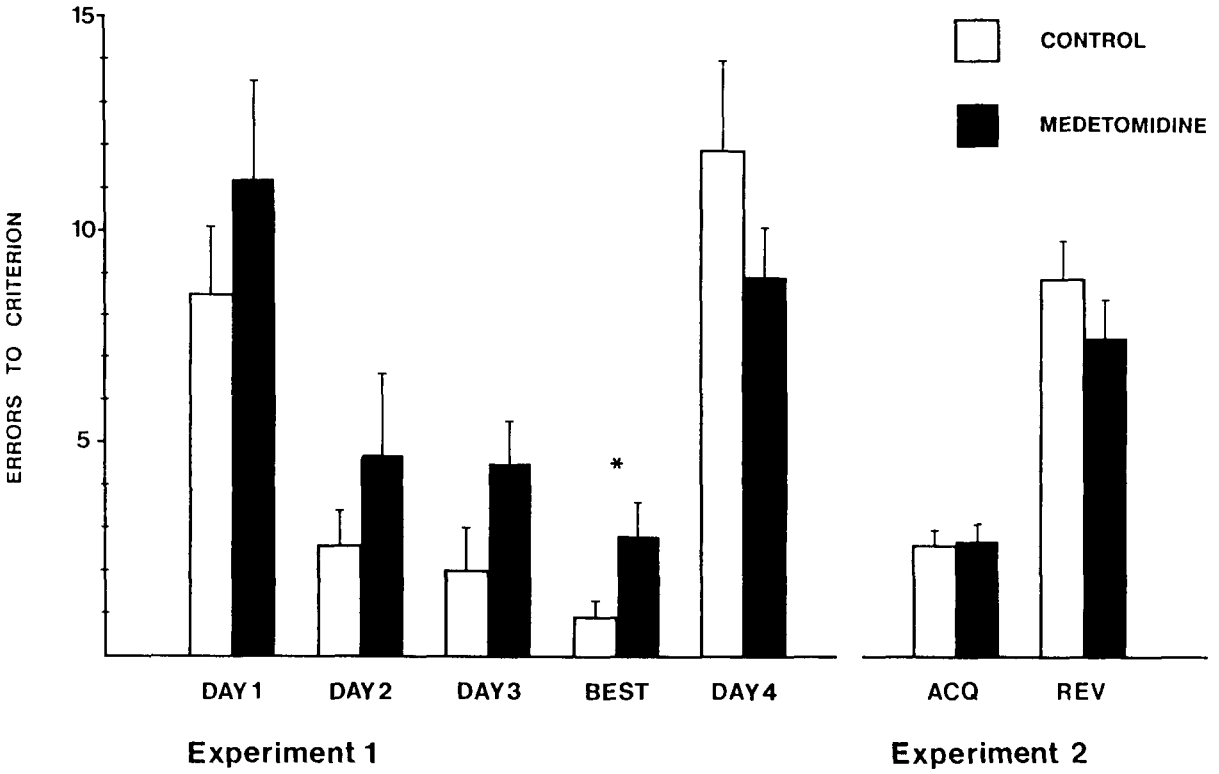

FIG. 1. Acquisition and reversal of position discrimination. The bars indicate mean number of errors to criterion and the brackets SEMs. The columns from left to right: 1-3, days of acquisition; 4, best achieved performance level (day 2 or 3); 5, reversal on the fourth day of Experiment 1; 6, acquisition; 7, reversal of Experiment 2. Open bars, control group; solid bars, medetomidine group. *$p < 0.05$, analysis of variance.

ference between days 2 and 3 was insignificant, $t_{dep}(18) = 0.37$, $p > 0.70$. Five rats (one male and one female in the medetomidine group, one male and two female controls) performed even worse on day 3 than on day 2. Rats in the medetomidine group tended to learn the place discrimination more poorly than controls; the treatment main effect was marginally significant, $F(1, 15) = 3.9$, $p = 0.07$ (Fig. 1). Considering the best performance irrespective of the day, controls made significantly fewer errors to the criterion than medetomidine-treated rats, $F(1, 15) = 6.1$, $p = 0.03$ (Fig. 1). There was a marginally significant sex × treatment interaction, $F(1, 15) = 3.9$, $p = 0.07$, males in the medetomidine group being worse than other rats. In the reversal phase of Experiment 1, females in the medetomidine group made fewer perseverations than female controls ($U = 2.0$, $p = 0.03$), but no treatment effect was found in males (Fig. 2). In Experiment 2, there was a significant session main effect both on acquisition, $F(1,15) = 4.7$, $p = 0.05$, and reversal, $F(1, 15) = 5.0$, $p = 0.04$, rats being worse in the acquisition but better in the reversal on the second session. No significant treatment effects were found.

In Experiment 1, the rank correlation between the best performance in position discrimination and the reversal learning was insignificant both in the saline ($r = 0.04$ for errors, $r = -0.07$ for perseverations) and medetomidine groups ($r = -0.32$ for errors, $r = -0.08$ for perseverations). In Experiment 2, there was an almost significant negative rank correlation between acquisition and reversal in the saline group ($r = -0.40$ for errors, $r = -0.44$ for perseverations, $p = 0.05$) but not in the medetomidine group ($r = -0.11$ for errors, $r = -0.17$ for perseverations). The dependence of the error scores upon the behavioral parameters is presented as an intercorrelation matrix separately for the control and medetomidine groups in Table 1.

DISCUSSION

The present data suggest that a low dose of medetomidine slightly impairs the reference memory of aged rats regardless of sex. Medetomidine-treated rats reached a lower level of accuracy in their position discrimination performance than controls, but the differences between groups in the rate of acquisition did not reach significance. This is in line with the finding that a low dose of guanfacine slightly impaired the performance of aged, female rats in the Morris water maze task, considered a test for reference memory (23).

The main finding of the present study was that medetomidine reduced the number of perseverations in the reversal of an established habit, but only in females. This cannot be ascribed to a more poorly learned position habit of medetomidine-treated rats because there was no correlation between their error scores in the position discrimination and their number of perseverations in the reversal. Although the first trials in the reversal tax working memory, it is not likely that the decrease in perseverations was due to improved working memory. First, the medetomidine group was inferior to controls during the first learning session, which taxes working memory as much as the reversal learning. Second, the effect of medetomidine reached significance in the reversal of Experiment 1

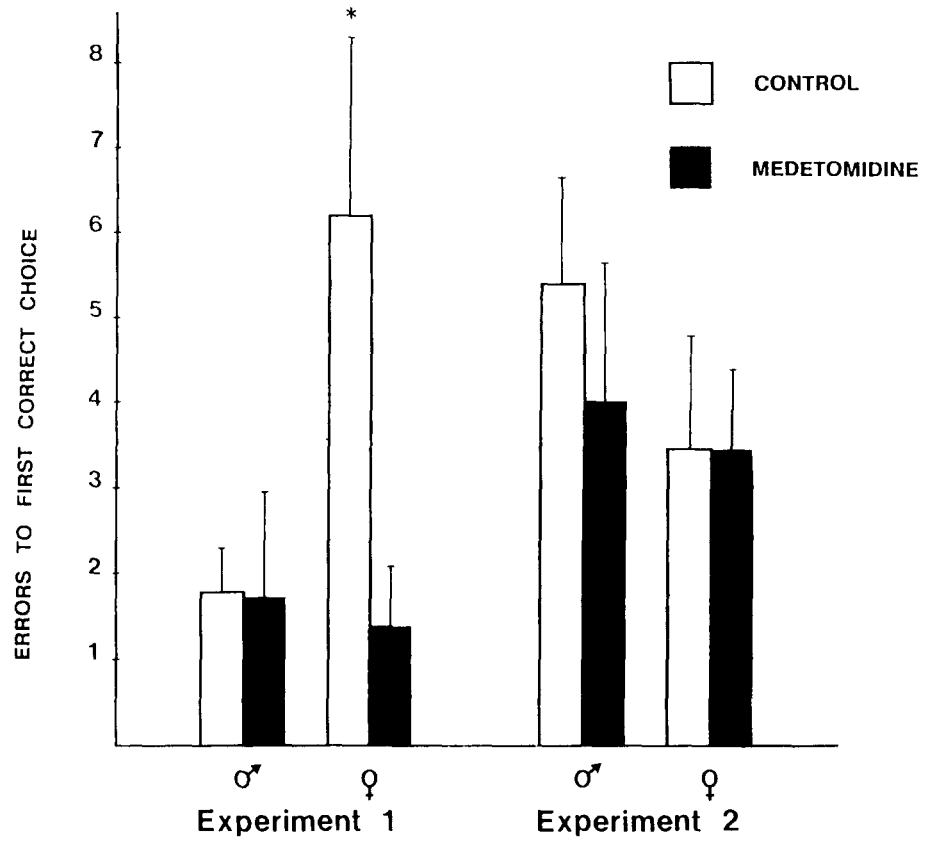

FIG. 2. Perseveration in the reversal phase of Experiment 1 (separate session) and reversal 2 (same session). The bars indicate mean number of errors to the first correct choice after reversal and the brackets SEMs. The data is shown separately for both sexes. Open bars, control group; solid bars, medetomidine group. *$p < 0.05$, Mann-Whitney U-test.

but not in the reversal in the same session (Experiment 2) although the latter makes stronger demands on working memory. The fact that medetomidine reduced perseveration only in the reversal of a well-established habit is in line with the notion that depletion of forebrain noradrenaline retards the extinction of the nonrewarded response in an appetitive task (19,20). It is tempting to speculate that aged, female rats have a more degenerated noradrenergic system than males and therefore are more resistant to extinction, but this remains to be studied.

An alternative explanation to both the slightly impaired acquisition of position habit and the improved reversal learn-

TABLE 1

SPEARMAN RANK CORRELATIONS BETWEEN BEHAVIORAL PARAMETERS (AMBULATIONS AND REARINGS IN OPEN FIELD, DAYS TO START EATING IN THE MAZE) AND ERROR SCORES IN EXPERIMENTS 1 AND 2

	Control			Medetomidine		
Test Paramater	Ambulations	Rearings	Eating	Ambulations	Rearings	Eating
First session 1	−0.08	0.41	0.45	−0.25	−0.10	0.06
Best performance 1	0.03	0.07	0.05	−0.70*	−0.48	0.71*
Reversal 1	0.58	0.61	0.07	0.23	0.22	0.37
Perseveration 1	0.81†	0.37	−0.06	−0.01	−0.33	−0.10
Acquisition 2	0.34	0.59†	0.01	−0.10	−0.30	−0.19
Reversal 2	0.08	−0.07	−0.28	0.04	0.23	−0.18
Perseveration 2	−0.10	−0.05	−0.32	−0.13	−0.01	−0.05

*$p < 0.05$.
†$p < 0.01$.

ing in medetomidine-treated rats is that the drug increased spontaneous alternation behavior. Decreased spontaneous alternation has been reported in both aged (30) and noradrenaline-depleted rats (20) and ascribed to decreased exploratory tendency (30) or increased neophobia (20). Few findings in the present study support this notion, however. First, females showed much more exploratory activity in the open field than males, but also most perseverations in the reversal. Second, in the medetomidine group there was a negative correlation between exploratory activity and the error score of the best performance in position discrimination. A major effect of neophobia on the maze performance is unlikely because of the extensive pretraining. Nevertheless, the correlation in medetomidine-treated rats between the high number of sessions needed to start eating in the maze and high error scores of the best performance in position discrimination suggests that the most neophobic individuals showed increased spontaneous alternation at least during early test sessions. This is in line with the documented anxiolytic effect of medetomidine (22).

The slightly impaired reference memory of both sexes and decreased tendency of aged, female rats to perseverate cannot account for the beneficial effect of medetomidine on the T-maze alternation performance observed in aged, male rats (9). It is likely that the cognitive demands are different in these two tasks despite some apparent similarities. In the present study, the memory function was tested during a learning process, while in the delayed alternation task it was tested in a well-learned paradigm. The task used in this study was relatively easy for rats to learn, whereas they needed several weeks of intensive training to master the delayed alternation task with varying delays and even then performed close to change level with the longest delays. It is possible that the cognitive effects of α_2-agonists in the aged brain vary depending upon the specific type and difficulty of the task.

It is most likely that the observed effects of medetomidine are mediated through the α_2-adrenoceptor, but it should be noted that medetomidine also binds to nonadrenergic imidazoline sites (11,29). In the rat, the prefrontal cortex, which has been shown to be important in spatial reversal learning (7,10), is almost devoid of imidazoline sites (13), but the hippocampus and neostriatum, which have also been shown to be involved in spatial reversal (7,14), are relatively rich in imidazoline binding sites (13). Further studies with direct intracerebral application of the drug are needed to elucidate the site and mechanism of action of medetomidine in the aged brain.

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