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BRIEF COMMUNICATION

Memory Retention Test Performance in Mice: Improvement by Chronic Oral Cholinergic Drug Treatment

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FLOOD, J. F., G. E. SMITH AND A. CHERKIN. *Memory retention test performance in mice: Improvement by chronic oral cholinergic drug treatment.* PHARMACOL BIOCHEM BEHAV **21**(1) 169–173, 1984.—Mice consumed solutions containing 0, 0.025, 0.050 or 0.075 mg/ml of arecoline hydrobromide (ARE) one week prior to training (T-maze, footshock, active avoidance) and a total of two weeks prior to testing memory retention. The mean daily doses of ARE were estimated to be 0, 157, 302, or 500 μg per mouse, respectively. An inverted-U dose-response curve was obtained; the best retention test performance was by the group receiving 0.050 mg/ml of ARE. Measures of activity and weight taken over the experiment indicated no significant differences between ARE groups and the control group; thus no apparent toxicity. Separate groups of mice consumed 0 or 0.050 mg/ml of ARE for one week, then were trained to a criterion of 5 avoidances in 6 training trials. There were no significant differences in trials to first avoidance response or to criterion. Thus the enhanced retention test performance of the 0.050 mg/ml ARE group reflected improved memory processing rather than better learning.

Alzheimer's disease Arecoline Cholinergic Chronic Dementias Memory Mice Retention

A long-range goal of studying drug effects on memory processes is rehabilitation of memory failure due to disease and aging. Over the next two decades, a sizeable increase in the incidence of Alzheimer's disease and senile dementia is expected [13]. Dementia's most consistent symptom is the loss of memory [12]. Acute administration of cholinomimetics improves memory retention [1, 2, 3, 6, 10, 11]. A basic assumption of this research and a rationale for its clinical application is that an increased level or duration of neurotransmitter activity alters memory processing. We recently confirmed the hypothesis that increasing the duration of cholinergic activity will improve memory processes, by administering intracerebroventricularly 1, 2, or 3 successive injections of a cholinergic agonist, arecoline, at 90-min intervals starting immediately after training [7]. Pharmacological alleviation of memory disorders necessarily involves the prolonged use of drugs. Since most drug research on memory has used acute administration, a question arises as to whether such research will provide information that is pertinent to the prolonged drug treatment needed to improve memory in those afflicted with memory disorders. We now report the facilitation of memory retention in mice by chronic oral ingestion of arecoline over a two-week period.

METHOD

Subjects

The subjects were 8-week old C57B1/6Ncr male mice. Only subjects weighing between 22 and 25 grams were used. The mice were individually housed throughout the experiment. The drinking water was provided in a 50-ml centrifuge tube (Nalgene) and a stopper with a dripless spout (ATCO Mfg.). The tubes contained a vehicle of 0.02% sodium saccharine and 0.0015% methyl salicylate (oil of wintergreen) in distilled water, to mask any unpleasant taste of arecoline in the solution. During the first week, mice were provided with the vehicle continuously. During the following 3 weeks, arecoline hydrobromide (ARE; Sigma Chemical Co.) was added to the solution for the experimental groups. Drug or vehicle solutions were available at all times during three-week treatment period, with fresh solutions provided at 2–3 day intervals. The mice showed no behavioral signs of toxicity such as diarrhea, salivation, shakes or tremors.

Training

The mice were partially trained to avoid footshock (0.30 mA) in a T-maze constructed of black plastic. The maze

consisted of an alley with a start box and guillotine door at one end and two opposed goal boxes at the other end of the alley. A shock grid floor ran throughout the maze. A mouse was placed into the start box on the first training trial, the guillotine door was raised and simultaneously a buzzer sounded, followed 5 sec later by continuous scrambled footshock. The goal box which the mouse first entered was designated "incorrect" and the footshock continued until the mouse entered the opposite goal box. On all subsequent training trials, the latter goal box was "correct" for a given mouse. On the next three training trials, the mouse was placed in the start box, the buzzer was sounded as the guillotine door was raised, and a 5-sec non-shock interval was allowed for the mouse to reach its correct goal box and thereby avoid footshock. If the mouse did not reach the correct goal box in 5 sec, it received footshock until it did so.

Retention Testing

One week after training, retention for T-maze training was tested by continuing training as described above until the mouse made an avoidance response. An avoidance response occurs when a mouse enters the correct goal box in 5 sec or less. Mice were trained only until they made one avoidance response (except in Experiment 2) because in this apparatus mice generally continue to make a string of avoidances after making the first avoidance. In Experiment 2, where drug effects on acquisition are being measured, the mice were trained until they made 5 avoidances in 6 trials. Even the naive subjects averaged slightly more than 1 escape trial in reaching criterion. Thus in Experiment 1, training to a higher criterion than the first avoidance response would not have provided any better measure of retention. Two measures of retention were used: (a) the number of trials to make the first avoidance response and (b) the percent of mice classed as remembering (Percent Recall Score). Mice making one avoidance response in three trials or less were classed as remembering the original training. This criterion was used because it provided optimal differentiation of the performance of naive versus well-trained mice [4].

Activity Measurement

Spontaneous activity was measured in an activity wheel (15 cm in dia) placed inside a test chamber (25×38×27 cm), and monitored automatically by infrared photocells. The mice were given 10 min each day to explore the test chamber. During the next 20 min activity in the wheel was recorded; however, the mice could spend the time exploring the test chamber rather than run in the wheel. The tests were given on three consecutive days following retention testing. The drug conditions were continued during this period.

Statistical Analysis

Two measures of memory retention were analyzed. The first measure was the mean number of trials to the first avoidance response, for all subjects within a group. The overall significance of the drug treatment effect was determined by ANOVA. Dunnett's t-test was used to test the significance of a drug group mean against its control group mean. To make the nonorthogonal comparisons between drug group means, Tukey's test was used. The second measure, percent recall score, was derived in order to visualize better the effects of drug treatments on retention. Those subjects making their first avoidance in three trials or less were classed as remembering the original training. The nonparametric Fisher exact probability test was used to test for significant differences among percent recall scores.

EXPERIMENT 1: EFFECTS ON MEMORY RETENTION

The purpose of this experiment was to determine if chronic oral ingestion of ARE would enhance memory retention as it did when administered acutely, either intracerebroventricularly [5,6] or subcutaneously [8]. The subjects and housing were as described above. During the first week, the mice were provided with the vehicle so that they would habituate to the novel taste and so that we could obtain a baseline of each mouse's pattern of fluid consumption. After the baseline period, the subjects were assigned to groups as follows. The control group was assigned those mice which drank the most (top 10%) and the least (bottom 10%). In addition, mice were assigned to this control group whose mean daily consumption differed by more than 1 ml across the 7-day baseline period. These mice were assigned to the control group since their inclusion in the drug groups could confound the anticipated dose-response relationship of the study by their consuming significantly more or less than the mean. The remaining subjects were divided among the three drug groups and control so that the mean consumption and standard deviation were as similar as possible. The control group had a higher standard deviation but the mean fluid consumption was about equal to those of the other groups. During the second week and until the end of the experiment the control group continued to receive the vehicle, while the three other groups received ARE (0.025, 0.050 or 0.075 mg/ml) added to the vehicle. To obtain an estimate of the amount of drug ingested, fluid consumption was monitored throughout. Based on the mean fluid consumed (Table 1), the corresponding daily doses of ARE were 157, 302 and 500 μg per mouse. Based on the mean fluid consumption and the mean body weight during the experiment the dose was 6, 12, and 20 mg/kg/day, respectively. At the end of the second week, the mice were partially trained on T-maze active footshock avoidance as described. At the end of the third week, training was continued until a mouse made an avoidance response. After the retention test, activity of the various groups was measured in activity wheels for a 20-min period on each of three consecutive days and then the final weight of each mouse was recorded. The mean weight gain over the 4-week interval was calculated for each group.

Results

The control vs. drug condition was significant at $p<0.01$, ANOVA $F(3,86)=4.62$. As expected, control retention was poor, with the mean trials to first avoidance being 4.6 and with a recall score of 20%. Mice receiving 0.050 mg/ml ARE (ARE-0.05) in their drinking water showed the best retention, with a mean of 3.05 trials to first avoidance and with a recall score of 80% (Table 1). The difference between the control group and ARE-0.05 was significant at $p<0.01$ (Dunnett t-test). The other groups received either not enough ARE in the vehicle (ARE-0.025) or too much (ARE-0.075), for maximal retention scores. The ARE-0.05 group had significantly higher scores than the ARE-0.075 group ($p<0.05$, Tukey's t-test) indicating that ARE-0.075 was amnestic relative to ARE-0.05. This replicates the inverted-U dose response curves previously reported for ARE with other routes of administration [5, 6, 8]. Fluid consumption was stable across

TABLE 1

EFFECT OF CHRONIC INGESTION OF ARECOLINE ON RETENTION TEST PERFORMANCE

	Control	ARE (0.025 mg/ml)	ARE (0.050 mg/ml)	ARE (0.075 mg/ml)
N/Group	30	20	20	20
Mean Trials to 1st Avoidance	4.53	3.80	3.05*	4.25†
S.E.M.	0.26	0.34	0.24	0.41
% Recall Score	20	35	80‡	30
Mean Fluid Consumed (ml)	6.37	6.14	6.03	6.56
S.D.	0.85	0.75	0.71	0.62
ARE Consumed (mean μg/mouse/day)§	0	157	302	500
ARE Consumed (mean mg/kg/day)	0	6	12	20
Mean Weight Gain (g)	5.28	5.95	4.75	5.06
S.D.	1.14	1.41	1.50	0.74
Activity Score (%)¶				
Day 1	17	20	20	15
Day 2	55	60	60	50
Day 3	70	70	80	70

*Only ARE (0.050 mg/ml) differed significantly in mean trials to 1st avoidance response from the control group, $p<0.01$ (Dunnett t-test).

†Among the drug treated groups, the only significant differences in mean trials to 1st avoidance response was between ARE (0.050 mg/ml) and ARE (0.075 mg/ml), $p<0.05$ (Tukey's t-test).

‡ARE (0.050 mg/ml) differed significantly in recall score from the control group, $p=0.0002$ (Fisher Exact Test).

§Based on mean body weight between the start and the end of the Experiment (3 weeks).

¶Activity counts yielded bimodally distributed data. Activity counts greater than 100 were considered normal in that injections of psychomotor drugs like amphetamine or scopolamine result in scores of less than 100. The percent of subjects showing normal activity is tabled.

the entire experiment and variability among subjects in a group from day to day was low. Neither activity level in the activity wheel nor weight gain showed any significant differences among groups (Table 1).

EXPERIMENT 2: EFFECT OF ARE ON ACQUISITION

The results of Experiment 1 could be interpreted as meaning that the group which consumed 0.05 mg/ml of ARE in their drinking solution learned better and therefore their retention test performance was superior to the control group's. The purpose of Experiment 2 was to determine if ARE at 0.05 mg/ml facilitated acquisition of the T-maze footshock avoidance habit.

The subjects and housing were the same as in Experiment 1. Only two groups were used: control and ARE-0.05. The training was as described above except that all mice were trained until they made 5 avoidance responses in 6 training trials. The trial of the first avoidance response and the trial on which criterion was reached were recorded. No retention test was given after this level of training since retention would be uniformly high in both groups. After a one-week baseline period with the vehicle, subjects were assigned to the control or ARE-0.05 drug condition as described above. The drug treatment preceded training by 1 week, as in Experiment 1.

Results

The omnibus F of the ANOVA was not significant ($F<1$) indicating that the mice that had 0.05 mg/ml of ARE added to the vehicle did not learn to make avoidance responses faster than control subjects by either of two measures: mean trials to first avoidance response or mean trials to the 5 out of 6 criterion (Table 2).

DISCUSSION

Reasonable uniformity in the volume of fluid consumed is essential in this paradigm, since the drug dose is proportional to the fluid consumed. Table 1 shows that over the duration of the experiment the standard deviation of fluid consumption ranged from 9% of the mean (ARE-0.075) to 12% of the mean (ARE-0.025). Part of this variability was probably due to weight differences within groups and part was due to in-

TABLE 2
EFFECT OF ARE ON ACQUISITION

	Control	ARE (0.05 mg/ml)
Mean Trial of First Avoidance	5.55	5.45
SEM	0.34	0.32
Mean Trials to 5/6 Criterion	10.20	10.35
SEM	0.36	0.27

creased fluid consumption associated with an increase in weight over the period of the experiment. Differences in activity levels between drug groups and the control group could affect performance on the retention test. In previous work, we found that low doses of psychomotor stimulants such as amphetamine (2 mg/kg, SC) or scopolamine (1 mg/kg, SC) resulted in *decreased* wheel running (e.g., less than 100 counts per 20 min test) and increased general activity (unpublished results). Table 1 shows that the percentage of mice showing normal activity levels (greater than 100 counts) did not differ between the control and the drug groups. The results across days also indicate that motor learning was not affected by the drug treatment since the degree of improvement (increase in percent of subjects with counts greater than 100) was about the same across groups. Thus, neither altered activity nor better motor learning accounts for the enhanced retention test performance of the ARE-0.05 group.

Enhanced acquisition during original training could have accounted for the superior retention test performance of the ARE-0.05 group. Table 2 indicates that neither on a measure of trials to first avoidance nor trials to a 5 out of 6 avoidance criterion were there significant differences. Thus, enhanced acquisition does not explain the facilitated retention test performance of the ARE-0.05 group.

The generality is now established of arecoline's ability to improve retention test performance whether administered acutely (intracerebroventricularly, subcutaneously) or repeatedly (intracerebroventricularly, orally). In addition, our previous studies used a randomly bred, white male albino mouse strain (CD-1) while this study used an inbred, black male mouse strain (C57B1/6Ncr). Thus improvement of retention test performance with ARE was not dependent on the use of a particular strain of mice. The amnestic effect of 0.075 mg/ml of ARE in the drinking solution as compared to 0.050 mg/ml of ARE was not associated with signs of toxicity, since neither activity nor weight gain was affected. The inverted-U dose response curve found is characteristic of cholinomimetics, regardless of route of administration [5, 6, 8].

In a recent report [9], 15 days of chronic subcutaneous administration (by Alza minipumps) of physostigmine impaired retention for one-trial passive avoidance tested 24 hr after training. Scopolamine given under the same conditions improved retention. Of the several differences between that study and the one we are reporting, terminating the drug treatment 24 hr prior to training, and testing only a single dose of each drug, may account for differing results. The rationale for terminating the drug treatment prior to training and testing was that chronic anticholinesterase treatment would result in a decrease in the muscarinic receptor density and chronic anticholinergic treatment would result in an increase in the density of muscarinic receptors. Thus at the time of training the decreased density of receptors, assumed to result from chronic infusion of physostigmine, would impair learning and memory while an increased density of receptors resulting from chronic scopolamine treatment would enhance learning and memory.

We conclude that chronic oral treatment of the mice with 0.050 mg/ml of ARE in their drinking solution improved memory processing and not retention test performance *per se*. Additional research will need to be done to determine the generality of the effect across cholinomimetics and the possible confounding influence of changes in receptor density and sensitivity with chronic drug administration. Other research in progress will determine the degree to which improved retention exists when other training tasks are used and the degree to which such treatments can have antiamnestic effects against amnesias resulting from drug administration, surgical insult to the brain or old age.

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