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Attenuation of Forgetting by Pharmacological Stimulation of Aminergic Neurotransmitter Systems

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QUARTERMAIN, D., M. E. JUDGE AND P. LEO. *Attenuation of forgetting by pharmacological stimulation of aminergic neurotransmitter systems.* PHARMACOL BIOCHEM BEHAV **30**(1) 77–81, 1988.—Mice were trained in one-way active avoidance to a criterion of 3/4 avoidances and tested under extinction conditions one week later when substantial forgetting had occurred. Thirty min prior to testing animals were injected with either saline or different doses of drugs which activate the noradrenergic (phenylephrine, salbutamol, clonidine) dopaminergic (L-dopa(Sinemet) transdihydrolisuride, apomorphine) and serotonergic (fluoxetine, 5-methoxy DMT) neurotransmitter systems. Results showed that all agents alleviated forgetting in a dose dependent fashion. Untrained mice treated with the most effective dose of representative drugs from each class did not exhibit avoidance behavior at testing indicating that the improved performance of trained animals was probably not the result of increased activity or other non-memorial effects of the drugs. It was concluded that pharmacological agents which stimulate monoamine systems may improve memory retrieval by activating a non-specific neural system which controls arousal, attention and motor readiness.

Memory retrieval Forgetting Active avoidance Memory facilitation Biogenic amines Memory processing

THE possibility of improving memory by the use of pharmacological agents is attracting increasing attention. Much of the current interest in this topic can be traced to the need to find treatments for the incapacitating memory dysfunctions which result from diseases of the central nervous system as well as the more benign impairments which accompany normal aging. Much of the research in this area has focussed on testing the efficacy of drugs which increase activity in the cholinergic system principally because low levels of markers of cholinergic function had been found in the brains of patients who died from Alzheimer's disease. The results of attempts to improve disordered memory with cholinergic agents have so far been disappointing and it may now be an appropriate time to determine whether pharmacological agents which influence other transmitter systems can improve human memory disorders.

A large body of literature exists which indicates that monoamine neurotransmitter systems play an important role in regulating memory function in animals (see [7,10] for reviews). Several studies from this laboratory have shown that drugs which activate norepinephrine and dopamine neurotransmitter systems can improve retention of several different learned responses in rodents when they are administered prior to testing (e.g., [1, 2, 6, 12, 15]). These pre-test treatments do not merely improve performance by non-specifically altering activity or by increasing levels of motivation. Rather they appear to selectively facilitate the retrieval of stored information [11, 13, 14]. These studies have tested a relatively narrow range of aminergic agents (most commonly d-amphetamine) in tasks where the retrieval enhancing potential has been evaluated by the capability of the drug to ameliorate experimentally-induced amnesias (most frequently produced by protein or catecholamine synthesis inhibition). In order to establish that aminergic agents may have potential for improving human memory it will be necessary to demonstrate that the drugs can alleviate memory loss caused by a variety of forgetting treatments.

The intention of the present study is to examine the retrieval enhancing capability of a number of pharmacological agents which activate the monoamine neurotransmitter systems norepinephrine (NE), dopamine (DA) and serotonin (5-HT). In these experiments forgetting will be induced by a one week training to testing interval rather than by the use of standard amnestic agents. We believe that the alleviation of spontaneous forgetting may be a more reliable indication of a drug's potential for improving human memory than the reversal of amnesia caused by disruption of a specific neurochemical system.

METHOD

Subjects

The subjects for this study were male Swiss Webster mice

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(Taconic, NY) 10 weeks of age and approximately 35 grams body weight. Animals were housed 4 per cage with food and water ad lib.

Behavioral Task and Apparatus

Retention of one-way active avoidance was employed to assess the retrieval enhancing capacity of the selected pharmacological agents. Mice trained to a relatively weak criterion (3/4 avoidances) show good retention 1 day following training but exhibit substantial forgetting after 1 week. Drugs are administered prior to the 1 week test.

Animals were trained in a two compartment avoidance apparatus consisting of an aluminum V trough and a black Plexiglas "safe" chamber. The dimensions of the trough and the safe chamber were 5×5×4 in. and the compartments were separated by a guillotine door. The safe chamber was covered with a black Plexiglas lid while the V trough was covered with a clear acrylic sheet painted white except for a 5×5 cm area above the door which was left clear for observation. A 5 watt miniature lamp was mounted in the center of the lid. The walls of the trough were made from 2 mm sheet aluminum, the bottom 10 cm of which was bent inwards at a 150 degree angle so that the floor consisted of a V shaped trough made up of the two wall plates suspended at 50 degrees from each other. The plates were held 0.5 cm apart at the bottom to allow urine and feces to escape. Each of the walls was connected to one pole of a Grason Stadler constant current shocker (model No. 700) which was activated by control circuitry to deliver 0.6 mA AC shocks.

Procedure

Training. Training was initiated by placing the mouse in the V trough facing away from the door. After 5 sec the CS (flashing light 0.5 sec on; 0.5 sec off) was activated and the door raised starting the latency timer. After 10 sec the UCS was automatically initiated and the CS, UCS and latency timer were simultaneously terminated when the animal crossed into the "safe" compartment. Mice were removed and transferred to a holding cage for the 30 sec intertrial interval (ITI). Training was terminated when mice attained a criterion of 3/4 avoidances.

Testing. Retention testing was carried out under extinction conditions. Mice were placed in the V trough and the same procedure employed during acquisition was used except that no UCS was scheduled. Animals failing to cross into the black compartment within 60 sec were given the maximum latency as a test score. Five trials separated by a 30 sec ITI constituted the test procedure for this task.

Pharmacological Treatments

Animals were injected subcutaneously 30 minutes before the retention test with one of the drugs listed below. Injections were adjusted for individual animals by injecting 0.01 ml per gram body weight. Drug solutions were prepared so that the drug quantity required for 1 kg of body weight was dissolved in 10 ml of saline solution. The following substances were tested:

(a) Physiological saline,
(b) Phenylephrine hydrochloride (Sigma) 0.01, 0.1, 1.0 mg/kg,
(c) Clonidine hydrochloride (Boehringer Ingelheim) 0.1, 0.5 mg/kg,
(d) Salbutamol sulphate (Sigma) 0.1, 1.0 mg/kg,

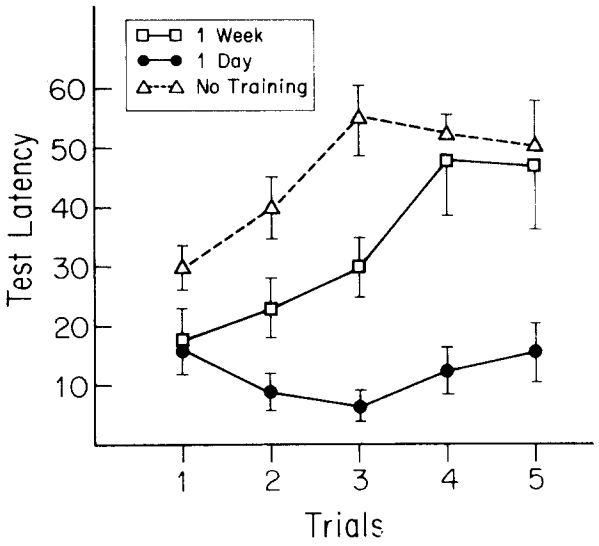

FIG. 1. Mean latencies on the 5 test trials for animals tested 1 and 7 days after avoidance training.

(e) Apomorphine hydrobromide (Merck) 0.1, 2.0, 4.0 mg/kg.
(f) Transdihydrolisuride (Schering) 0.1, 0.2, 0.5 mg/kg,
(g) L-Dopa (Sinemet, Merck Sharp and Dohme) 10, 20 mg/kg,
(h) 5-Methoxy-N,N-dimethyl-tryptamine (Sigma) 0.1, 1.0, 5.0 mg/kg,
(i) Fluoxetine hydrochloride (Eli Lilly) 2.0, 5.0 mg/kg.

The drugs were selected to activate principally norepinephrine (b,c,d), dopamine (e,f,g) and serotonin (h,i) neurotransmitter systems. The dose levels selected for studies were based on preliminary experiments and other published data from this laboratory.

EXPERIMENT 1: SPONTANEOUS FORGETTING OF ACTIVE AVOIDANCE LEARNING

Procedure

Nineteen mice were trained to a criterion of 3/4 avoidances using the procedures described above. One group (N=8) was tested 1 day and a second group (N=11) was tested 7 days following training. An additional group of mice (N=13) which had not received any training were tested at the same time as the 7 day group. The performance of the untrained group served as a baseline against which the magnitude of memory loss of the 7 day group could be compared.

Results

The mean latencies for the 5 retention test trials for the 3 groups is shown in Fig. 1. An ANOVA carried out on these data indicated a significant difference among the three groups, $F(2,145)=36.52$, $p=<0.001$, and a marginally significant effect of test trials, $F(4,145)=2.36$, $p=<0.0553$. The interaction between groups and test trials was not significant ($F=1.19$). Post hoc comparisons revealed that: (1) latencies of the 7 day group were significantly longer than those of the 1 day group, $F(1,85)=17.51$, $p=<0.001$, and (2) latencies of the group tested 1 week after training were significantly shorter than those of untrained mice, $F(1,110)=14.05$, $p=<0.001$. These results show that the training parameters

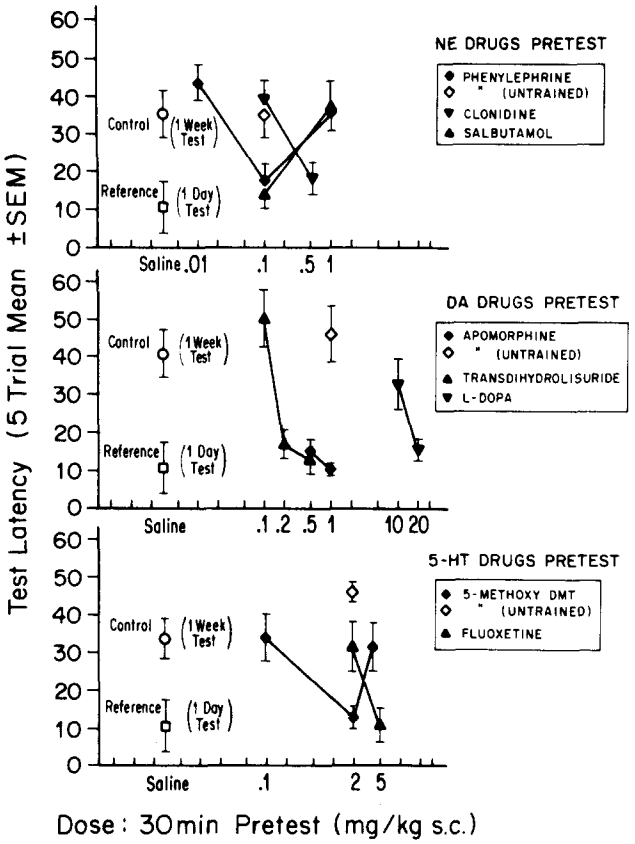

FIG. 2. Memory enhancing effect of aminergic compounds. Data are expressed as the mean of the 5 test trials and are plotted on a logarithmic scale. Reference groups represent non-injected animals tested 1 day after training. Separate 1 week saline control groups were run for each class of compound. Extent of alleviation of forgetting was determined by reference to this group.

employed in this study result in good retention 1 day post training but substantial forgetting after 1 week. In addition, they show that the 7 day group does not exhibit "complete" forgetting.

EXPERIMENT 2: EFFECT OF SPECIFIC RECEPTOR AGENTS ON RETRIEVAL

Procedure

This experiment was run in three sections; in section 1, different groups of animals were treated with either saline, N=11, salbutamol, 0.1 (N=12), 1.0 (N=11) mg/kg, phenylepherine, 0.01 (N=11), 0.1 (N=11) mg/kg or clonidine, 0.1 (N=12), 0.5 (N=10) mg/kg. In the second section, mice were treated with either saline (N=11), transdihydrolisure, 0.1 (N=8), 0.2 (N=11), 0.5 (N=12) mg/kg, apomorphine, 0.5 (N=12), 1.0 (N=12) mg/kg or L-dopa (Sinemet), 10 (N=10), 20 (N=12) mg/kg. In the final section, mice were injected with either saline (N=11), 5-methoxy DMT, 0.1 (N=10), 2.0 (N=12), 4.0 (N=12) mg/kg or fluoxetine, 2.0 (N=9), 5.0 (N=9) mg/kg. In addition, one compound was selected from each section and the most effective dose administered to a group of untrained animals (N=10 per group) to check on possible non-specific effects.

Results

Retention scores for each group are presented as the mean and SEM of the 5 retention test trials. This summary statistic facilitates comparison among the various drug groups without resulting in significant loss of information. The data from each drug group plus the 1 week saline control are shown in Fig. 2. Data from control animals tested 1 day after training are also included to permit a comparison of drug-induced facilitation with a 1 day memory. The doses of each drug along with the appropriate 1 week saline control group were subjected to one-way ANOVA followed by post hoc tests to determine the effective dose levels for each drug class.

NE Drugs

There were significant differences among the doses of all the drugs tested in this section. For phenylephrine, $F(2,30)=4.08$, $p=<0.05$, the lower dose (0.1 mg/kg) produced significantly better retention than the saline control, $t=2.41$, $p=<0.02$, while the high dose (1.0 mg/kg) had no effect in alleviating the forgetting. Salbutamol also produced dose dependent effects on retrieval, $F(2,31)=5.54$, $p=<0.01$, performance was enhanced following the low (0.1 mg/kg) dose, $t=3.1$, $p=<0.01$, but not following the high dose. Clonidine also attenuated forgetting, $F(2,30)=4.79$, $p=<0.05$, but with this agent the higher dose (0.5 mg/kg) was more effective ($t=2.3$, $p=<0.05$ vs. saline) than the lower dose. This confirms previous reports from this laboratory [9]. Untrained mice treated with the effective dose of phenylephrine (0.1 mg/kg) failed to show decreased test latencies suggesting that non-specific (i.e., non-memorial) effects were unlikely to have produced the enhanced performance in the trained animals. This finding is congruent with other experiments from this laboratory which have consistently shown that pre-test drug treatments are only effective if animals have been previously exposed to the training contingencies (e.g., [5, 7, 10]).

DA Drugs

The results of separate ANOVAS indicated that all three agents alleviated forgetting. Apomorphine, $F(2,32)=18.6$, $p=<0.001$, was effective at both dose levels and transdihydrolisuride, $F(3,38)=11.98$, $p=<0.01$, attenuated forgetting at 0.2 and 0.5 mg/kg but not at 0.1 mg/kg. L-Dopa, $F(2,31)=9.09$, $p=<0.01$, improved memory when given at a dose of 20 mg/kg, $t=3.13$, $p=<0.01$, but was without significant effect at 10 mg/kg. Reference to Fig. 2 shows that the most effective dose of transdihydrolisuride did not decrease test latencies in untrained mice.

5-HT Drugs

Retrieval was also enhanced by both 5-HT agents. 5-Methoxy DMT, $F(2,32)=7.32$, $p=<0.01$, facilitated retention at a dose of 2 mg/kg, $t=4.3$, $p=<0.001$, but not at a dose of 4 mg/kg. Fluoxetine, $F(2,26)=5.3$, $p=<0.05$, on the other hand improved retrieval at 5 mg/kg, $t=3.2$, $p=<0.01$, but was ineffective at the lower dose. The 2 mg/kg dose of 5-DMT did not increase speed of responding when administered to untrained mice.

DISCUSSION

These results indicate that pharmacological agents which

activate monoamine transmitter systems can improve remembering following spontaneous forgetting. Although the effective doses for each drug are relatively high they produced no gross alterations in behavior and in all three drug groups untrained animals were unaffected by doses which markedly increased response speed in trained animals. With several drugs (e.g., phenylephrine, salbutamol, and 5-methoxy DMT) lower doses were more effective in facilitating remembering than higher doses. Dose response curves of this type are unusual in classical pharmacology but are relatively common in animal studies of memory enhancement (e.g., [3,4]). The mechanisms underlying this effect are unknown but may be related to the finding that behavioral regulation is more efficient with optimal rather than maximal levels of CNS functioning.

No inferences can be drawn from these results about the relative retrieval enhancing potency of agents acting on particular neurotransmitter systems. Any conclusions would be vitiated by differences in absorbtion, distribution and pharmacokinetics of the test drugs.

A notable feature of these findings is the absence of specificity; all of the drugs that were examined significantly improved retention. This is perhaps not surprising since all three transmitter systems have been shown to be involved in memory processing. The means by which monoaminergic agents improve retention is unknown. It seems likely that drugs which activate these systems influence remembering indirectly by increasing arousal, improving selective attention and facilitating motor readiness. The drugs from the three transmitter classes may have common effects on a non-specific neural system which regulates these behaviors. Thompson [16] has recently shown that lesions in a functionally interconnected system which includes portions of the basal ganglia, an area of the limbic midbrain and the brain stem reticular formation impair retention in a wide variety of tasks. This system is heavily innervated by monoamine neurotransmitters which makes it a likely candidate for the substrate through which drugs used in the present study facilitate remembering.

The results of this study suggest that stimulation of peripheral as well as central receptors can facilitate retrieval of partially forgotten information. Neither phenylephrine nor salbutamol readily enter the brain [5] yet both produce strong facilitation of retrieval suggesting that stimulation of peripheral adrenergic beta receptors is sufficient to alleviate forgetting in the present experiment. This finding confirms the results of an unpublished study from this laboratory in which both d-amphetamine and the peripheral analog 4-OH amphetamine alleviated forgetting of active avoidance learning produced by a 1 week retention interval. On the other hand, a previously published study reported that 4-OH amphetamine failed to alleviate forgetting of an inhibitory avoidance response induced by treatment with the protein synthesis inhibitor anisomycin [14]. It is possible that the capacity of peripherally acting drugs to attenuate forgetting may depend on both the nature of the forgetting and on the task which is forgotten. Treatments which typically induce large amounts of forgetting (like protein synthesis inhibition) may be less susceptible to attenuation by exclusively peripherally acting drugs than those forgetting treatments like that employed in the present study where more modest levels of memory loss are obtained. That task variables may be a factor in determining whether peripherally acting drugs facilitate retrieval is suggested by studies by Palfai and his colleagues [8,9]. They showed that amines administered peripherally shortly after training could attenuate reserpine-induced amnesia for a passive avoidance task but not for discriminated escape reversal learning. The importance of peripheral transmitters for modulating memory storage processes has been recognised for some time but much less is known about the role of peripheral systems in facilitating memory retrieval. This laboratory is currently examining this issue as well as the more general question of the extent to which the effectiveness of retrieval enhancing drugs is determined by the type and magnitude of the memory loss.

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