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Comparisons of the Effects of Guanethidine, 6-Hydroxydopamine and Diethyldithiocarbamate on Retention of Passive Avoidance

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PALFAI, T. AND T. J. WALSH. *Comparisons of the effects of guanethidine, 6-hydroxydopamine and diethyldithiocarbamate on retention of passive avoidance.* PHARMAC. BIOCHEM. BEHAV. 13(6) 805–809, 1980.—Depending on the dose levels, guanethidine and diethyldithiocarbamate produced time dependent amnesia for a one-trial passive avoidance task. 6-Hydroxydopamine given at any dose level or at any time interval before or after training did not result in retention deficits. The results were discussed in terms of the effects of these agents on peripheral biogenic amines and the possible role of these amines in memory formation.

Amnesia Memory Antiadrenergic Drugs Biogenic amines

A common strategy for describing neurobiological mechanisms of memory formation is to investigate the behavioral consequences of amnesia-inducing drugs and relate them to the drug's known mechanism of action. The rationale inherent in this approach is that a reliable correlation between the drug's pharmacodynamics and its effects on behavior will provide significant information about the biological processes involved in learning and memory. This experimental strategy, along with other lines of indirect support [2, 5, 43] have led to the hypothesis that the catecholamines, norepinephrine (NE) and dopamine (DA) are critically involved in memory formation [8, 18, 30].

Drugs that impair the synthesis, storage, turnover or degradation of NE or DA have been reported on numerous occasions to impair retention [4, 10, 19, 27, 28, 33, 34, 35, 39, 40] and the amnesic effects of these compounds have been typically attributed to their ability to disrupt a central amine-dependent phase of memory formation [1,11].

The apparent correlations between brain biogenic amines and behavior, however, might lead to premature inferences of causality [27,28]. Several publications from our laboratory suggest that depletion of peripheral catecholamines with either reserpine or syrosingopine might be sufficient to account for the amnesia induced by these drugs [28, 29, 41]. However, because the effects of these compounds on the catecholamines are only one of their several complex pharmacological consequences [30, 36, 37], other independent support for the role of peripheral catecholamines in memory should be demonstrated. The purpose of the present experiment was, therefore, to investigate the potential amnesic effects of several agents that deplete peripheral catecholamines by pharmacological mechanisms that are different from those of reserpine or syrosingopine.

EXPERIMENT 1

Guanethidine, a guanidine derivative is classified as an adrenergic neuron-blocking drug [20, 21, 26]. Because of its highly polarized molecules, it does not penetrate the brain and has no demonstrable effects on brain catecholamines following parenteral administration [6,17]. Izquierdo and his colleagues [14,31] have shown that it impairs both aversive and appetitive behaviors implying that peripheral catecholamines play an important role in memory formation.

Since the amnesic effects of reserpine and syrosingopine appear to be mediated by the peripheral antiadrenergic effects of these drugs [29,41], guanethidine might also produce amnesia peripherally albeit by a different mechanism than these rauwolfias. In the experiment below, we examined this possibility by investigating the potential time- and dose-dependent effects of guanethidine on retention of a passive avoidance response.

METHOD

Animals

Adult male albino mice, bred from CD-1 stock in our animal colony were used. The animals were housed in groups of four in Econo plastic cages in temperature (70°–72°F) and humidity (50–70%) controlled colony room. Food and water were available ad lib and a 12-hr light/dark cycle was in effect. The mice at the time of testing weighed between 30–40 g and were approximately 70 days old.

TABLE 1
LATENCIES FOR GROUPS TREATED WITH DIFFERENT DOSAGES OF GUANETHIDINE BEFORE OR FOLLOWING PASSIVE AVOIDANCE TRAINING

Guanethidine Treatment	N	Median	p vs Vehicle
10 mg/kg			
240 min pre	15	300	NS
120 min pre	17	288	NS
30 min pre	13	300	NS
0 post	15	49	<0.01
40 mg/kg			
240 min pre	14	300	NS
120 min pre	20	84	<0.05
30 min pre	12	102	<0.05
0 post	19	81	<0.05
Vehicle	10	300	

Apparatus

A step-through passive avoidance apparatus similar to that described by Jarvik and Kopp [15] was used. Briefly, the apparatus consisted of a Plexiglas-covered V-shaped trough which was divided by a guillotine door into a small illuminated start chamber and a larger darkened compartment. Panels of stainless steel formed the walls and floor of the trough and served to deliver an AC footshock from a Grason Stadler Model 700 Constant Current Shock Generator.

Procedure

Eight groups of mice were administered either 10 or 40 mg/kg guanethidine sulfate (Ismelin, CIBA) at one of four treatment-training intervals. The intervals were 240, 120 or 30 min prior to training or immediately (0-post) after training. Dosages and time intervals were chosen on the basis of the reported pharmacodynamics and time course of action of guanethidine [20]. A control group (N=10) received distilled water (drug vehicle) 120 min prior to the training trial.

Passive avoidance training consisted of a single trial. Each mouse was placed into the illuminated start chamber and following 60 sec, the guillotine door was opened and latency to step-through into the darkened compartment was electronically timed. Immediately following step-through (defined as passage of the hind limbs beyond the threshold), the door was closed and the mouse given a 1 mA footshock for 3 sec.

Retention tests were given 7 days following training; again the mouse was placed into the start box and 60 sec later, the door was opened and step-through latency recorded to an arbitrary maximum of 300 sec.

RESULTS

Guanethidine had no effect on initial step-through latencies during the training trial and produced no overt signs of behavioral depression or toxicity.

A Kruskal-Wallis analysis of variance indicated that guanethidine had a significant effect on retention performance, $H(7)=17.40$, $p<0.02$. The median step-through latencies which served as the index of retention, along with the results of post-hoc Mann-Whitney U tests are presented in Table 1.

As the table indicates, guanethidine impaired retention in a time- and dose-dependent manner. Retention was disrupted following the administration of 10 mg/kg guanethidine immediately after training or if 40 mg/kg were injected either 120 or 30 min before or immediately after (0-post) training. The results suggest that guanethidine, a peripherally active adrenergic agent, might induce amnesia by interfering with peripheral catecholamine-dependent processes during memory formation.

EXPERIMENT 2

The compound, 6-hydroxydopamine (6-OHDA), is a catecholamine-specific neurotoxin. It is preferentially taken up by adrenergic neurons where its cytotoxic action destroys the presynaptic terminal [16,38]. Following systemic administration, the drug produces long-lasting depletion of peripheral NE without altering the levels or functional integrity of central catecholamine processes. If, as the results of the previous experiments suggest, peripheral adrenergic mechanisms are necessary for the formation of long-term memory, then peripherally administered 6-OHDA should produce retention impairments for a passive avoidance response. In the experiment below, we examined the potential time- and dose-effects of 6-OHDA on retention of passive avoidance.

METHOD

Animals and Apparatus

Animals and apparatus are as described in Experiment 1.

Procedure

Twelve groups of mice were injected IP with either 25, 50 or 100 mg/kg 6-OHDA at one of four treatment-training intervals. These intervals were 1440, 240 or 30 min before or immediately after (0-post) avoidance training. The drug solution was prepared fresh on the day of the experiment by dissolving 6-OHDA bromide in distilled water containing 0.5% ascorbic acid as antioxidant. A control group (N=10) was injected with distilled water containing 0.5% ascorbic acid (drug vehicle) 240 min before training.

The passive avoidance training procedure and the retention test procedure was the same as in Experiment 1.

RESULTS

Mice injected with 6-OHDA at any time interval exhibited no overt signs of behavioral toxicity during either the training trial or the retention test. The initial step-through latencies of 6-OHDA-treated mice and vehicle-injected controls were comparable during the training trial. The highest dose of 6-OHDA (100 mg/kg), however, produced 17% mortality irrespective of the time of injection.

The Kruskal-Wallis nonparametric analysis of variance indicated no significant time- or dose-effect of 6-OHDA on retention of this behavioral task, $H(11)=5.43$, $p<0.10$. The step-through latencies of all 6-OHDA-treated groups during the retention test were not significantly different from the latencies of vehicle-injected controls. As shown in Table 2, all groups had median step-through latencies of 300 sec.

While the data reported here appear inconsistent with the

TABLE 2

LATENCIES FOR GROUPS TREATED WITH DIFFERENT DOSAGES OF 6-OHDA BEFORE OR FOLLOWING PASSIVE AVOIDANCE TRAINING

6-OHDA Treatment	N	Median	p vs Vehicle
25 mg/kg			
1440 min pre	19	300	NS
240 min pre	15	300	NS
30 min pre	11	300	NS
0 post	9	300	
50 mg/kg			
1440 min pre	20	300	NS
240 min pre	16	300	NS
30 min pre	13	300	NS
0 post	10	300	NS
100 mg/kg			
1440 min pre	15	300	NS
240 min pre	15	300	NS
30 min pre	12	300	NS
0 post	12	300	NS
Vehicle	10	300	

TABLE 3

LATENCIES FOR GROUPS TREATED WITH DIFFERENT DOSAGES OF DDC BEFORE OR FOLLOWING PASSIVE AVOIDANCE TRAINING

DDC Treatment	N	Median	p vs Vehicle
300 mg/kg			
240 min pre	12	300	NS
120 min pre	15	117	<0.05
30 min pre	10	295	NS
0 post	11	300	NS
700 mg/kg			
240 min pre	10	300	NS
120 min pre	11	300	NS
30 min pre	13	78	<0.05
0 post	12	300	NS
900 mg/kg			
240 min pre	11	300	NS
120 min pre	15	61	<0.01
30 min pre	14	70	<0.01
0 post	14	137	NS
Vehicle	10	300	

hypothesis that peripheral adrenergic processes may modulate memory processes, the interesting pharmacodynamics of 6-OHDA might offer an explanation. Mueller and his colleagues [24,25] reported that the adrenal medulla, which is resistant to the cytotoxic actions of 6-OHDA, increases the synthesis and release of catecholamines following 6-OHDA-induced sympathectomy. Further, Grewal and Kaul [9] reported a shift in the ratio of NE/E synthesis such that NE production increased 101% while E synthesis decreased 36%. These compensatory changes in medullary catecholamine synthesis along with the potential adrenergic denervation supersensitivity might be sufficient reasons why 6-OHDA did not produce amnesia. Clearly, further studies are needed to investigate the above hypothesis.

EXPERIMENT 3

Diethyldithiocarbamate (DDC) is a dopamine-β-hydroxylase (DBH) inhibitor which produces a marked but temporary depletion of NE in the brain and periphery [7,23]. The drug interferes with the activity of DBH, thereby blocking the conversion of DA to NE [3]. Because of this relative selectivity of action on NE synthesis, DDC has been an important tool in investigating the role of NE.

DDC has been shown to produce impairments in a variety of learning tasks [13]. While it is possible that these effects were mediated centrally, Haycock and his colleagues [12] found no correlation between the effects of DDC on brain NE or DA and subsequent retention performance and it was shown [22] that the DDC-induced retention impairments could be attenuated by subcutaneously-administered NE. The implications of these findings are that the action of DDC on peripheral adrenergic mechanisms may be sufficient to account for its amnesic effect. The experiment below investigated the dose- and time-dependent effects of DDC.

METHOD

Animals and Apparatus

Animals and apparatus are as described in Experiment 1.

Procedure

Twelve groups of mice were injected IP with either 300, 700 or 900 mg/kg diethyldithiocarbamic acid-sodium salt (DDC) at one of four treatment-training intervals. These intervals were 240, 120 or 30 min before or immediately after passive avoidance training. A control group was injected with distilled water, the drug vehicle, 120 min before the training trial.

RESULTS

Animals injected with DDC prior to training appeared sedated during the training trial and had significantly longer step-through latencies than controls. By the time of the retention test, however, no residual toxicity was evident. Median step-through latencies for the various treatment groups are presented in Table 3. The Kruskal-Wallis analysis of variance revealed a significant treatment effect, $H(12)=25.38$, $p<0.02$.

While at several time intervals the various dosages of DDC produced amnesia, examination of the interquartile ranges showed that the largest dose (900 mg/kg) administered 120 min before training produced the most consistent effects on retention. Finally, although the initial step-through latencies of DDC-treated animals might suggest a drug effect on non-associative factors during acquisition, subsequent ex-

periments in our laboratory have shown that the DDC-treated animals fully experience the training trial [42].

DISCUSSION

In a series of experiments the effects of different dosages of Gu, 6-OHDA and DDC were examined. Administered at various times before or after a passive avoidance training trial, Gu and DDC were able to produce the most reliable retention impairments when given 120 min before training. This time interval appears to be optimal for the appearance of the amnesic effects following not only the above antiadrenergic drugs but also following reserpine or syrosingopine [29]. This time interval may be necessary for the functional depletion of the catecholamine system.

Since at least syrosingopine and Gu affect only peripheral catecholamines albeit by different mechanisms, it is suggested that their amnesic effects are mediated via peripheral catecholamines. The implication of this hypothesis is that the presence of a peripheral sympathetic reaction following learning is essential for subsequent retention, probably by providing a modulating influence on the neurobiological events responsible for the formation of memory. Antiadrenergic drugs may impair retention of some forms of aversively motivated behavior by preventing or attenuating the animal's capacity for a normal sympathetic reaction which would follow an aversive stimulus. Experiments are currently underway that investigate these hypotheses.

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