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Peripheral Catecholamines and Memory: Characteristics of Syrosingopine-Induced Amnesia

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WALSH, T. J. AND T. PALFAI. *Peripheral catecholamines and memory: Characteristics of syrosingopine-induced amnesia.* PHARMAC. BIOCHEM. BEHAV. 11(4) 449–452, 1979.—The effect of syrosingopine on retention of a passive avoidance trial in mice was investigated. The drug given in doses of 2.5, 4.0 or 6.0 mg/kg 2 hr before training, but not when given 24 or 0.5 hr before or immediately after training, resulted in amnesia 7 days later. Dopamine or norepinephrine administered systemically 15 min before to 10 min after training was able to block the syrosingopine-induced amnesia. The role of peripheral catecholamines in memory formation was discussed.

Memory Amnesia Rauwolfia alkaloids Catecholamines Passive avoidance

MANY pharmacological studies indirectly implicate the catecholamines, norepinephrine (NE) and dopamine (DA), in memory storage processes [4, 15, 18]. Drugs, like alpha-methyl-para-tyrosine (αMpT) and diethyldithiocarbamate (DDC) which decrease catecholamine levels both centrally and peripherally by blocking their synthesis, produce time-dependent memory impairments [8, 9, 19]. The amnesic effects of these agents have been commonly attributed to their ability to disrupt a central amine-dependent phase of memory formation [1, 4, 8, 9, 15, 18].

Apparent correlations between brain biogenic amines and behavior need not mean a causal relationship. Recent data from our laboratory suggest that the peripheral effects of reserpine on catecholamines might be sufficient to account for the amnesic effect of this drug. We reported that whole brain levels of NE and DA following an amnesic dose of reserpine were not related to the probability of memory formation [14]. A dose of 2.5 mg/kg reserpine given either 24 or 2 hr prior to passive avoidance conditioning produced comparable depletion of brain NE and DA at the time of training: however, the drug produced amnesia only if it was administered 2–5 hr before training. In a subsequent study we reported data which demonstrated that systemic administration of NE or DA, amines that do not penetrate the blood brain barrier [21,22], was able to block the reserpine-induced amnesia [13]. Taken together, the results of these studies suggested that the effect of reserpine on peripheral catecholamines might be sufficient to account for the drugs' amnesic effect. The experiments reported here are an extension of this investigation. Syrosingopine is a reserpine analogue with almost exclusively peripheral action [11, 12, 16]. Here we examined the effect of this drug on retention of a passive avoidance task, and investigated whether systemically-administered NE or DA were able to block the syrosingopine-induced amnesia as well.

EXPERIMENT 1

The purpose of the first experiment was to establish a syrosingopine time and dose response curve for amnesia in the passive avoidance paradigm used in our laboratory.

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 and 40 g and were approximately 70 days old.

Apparatus

A step-through passive avoidance apparatus similar to that described by Jarvik and Kopp [10] 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

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Grason Stadler Model 700 Constant Current Shock Generator.

Procedure

Two-hundred forty mice were divided into 16 groups. Groups were administered 1.0, 2.5, 4.0 or 6.0 mg/kg syrosingopine either 24 hr, 2 hr or 30 min before or immediately following (0-post) passive avoidance training. The drug solution was prepared according to Ponessa and DeBoer [17] by mixing crystalline syrosingopine (Singoserp, Ciba) with 2 drops of Tween 80, 1% ascorbic acid and 10 cc of distilled water. 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 when 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

Animals treated with even the highest dose of syrosingopine failed to exhibit any overt signs of behavioral depression. However, intial step-through latency was slightly elevated in the 6.0 mg/kg 2 hr pre-group.

In comparing retention performance, a Kruskal-Wallis nonparametric analysis of variance indicated a significant treatment effect ($p<0.01$). 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 can be seen in Table 1, 2.5, 4.0 and 6.0 mg/kg syrosingopine given 2 hr before passive avoidance training produced a time-dependent amnesia for the task. The lowest dose (1.0 mg/kg) or other treatment-training intervals produced no effect on retention.

TABLE 1

RETEST LATENCIES FOR GROUPS TREATED WITH VARIOUS DOSAGES OF SYROSINGOPINE BEFORE OR FOLLOWING PASSIVE AVOIDANCE TRAINING

Syrosingopine Treatment	Median	N	p vs vehicle
1.0 mg/kg			
24 hr pre	300	15	NS
2 hr pre	300	13	NS
30 min pre	300	12	NS
0 post	300	14	NS
2.5 mg/kg			
24 hr pre	300	14	NS
2 hr pre	58	18	<0.01
30 min pre	300	15	NS
0 post	300	13	NS
4.0 mg/kg			
24 hr pre	300	12	NS
2 hr pre	63	17	<0.01
30 min pre	300	14	NS
0 post	300	14	NS
6.0 mg/kg			
24 hr pre	300	15	NS
2 hr pre	28	16	<0.001
30 min pre	300	14	NS
0 post	300	10	NS

EXPERIMENT 2

In the second experiment we examined whether DA and/or NE, given systemically, would counteract the syrosingopine effect on memory. Since these amines do not cross the blood brain barrier [21,22], the antagonism they produce would suggest a role for peripheral catecholamines in syrosingopine-induced amnesia.

METHOD

Animals and Apparatus

Animals and apparatus are as described in Experiment 1.

Procedure

Seventy-five mice received 2.5 mg/kg syrosingopine 2 hr prior to avoidance conditioning and a second injection of either 10 or 50 mg/kg DA or 0.5 or 0.75 mg/kg NE, 15 min before training. We previously reported that these dosages and time interval are effective in counteracting reserpine-induced amnesia for this behavioral task [13].

TABLE 2

EFFECTS OF DOPAMINE AND NOREPINEPHRINE ON SYROSINGOPINE-INDUCED AMNESIA

Group	N	Mdn STL	p vs Saline
Syro+Saline	17	49	<0.01
Syro+0.50 mg/kg NE	15	300	NS
Syro+0.75 mg/kg NE	14	290	NS
Syro+10 mg/kg DA	12	86	<0.01
Syro+50 mg/kg DA	17	300	NS
Saline	10	300	—

RESULTS

A Kruskal-Wallis nonparametric analysis of variance indicated a significant overall treatment effect ($p<0.001$). Group median retest latencies are presented in Table 2, along with a group by group comparison with Mann-Whitney U tests. Both 0.5 and 0.75 mg/kg NE and 50 mg/kg DA were able to block the syrosingopine-induced amnesia. The lower dose of DA (10 mg/kg), however, did not affect the syrosingopine amnesia.

EXPERIMENT 3

In the third experiment we examined the time-dependent effect of catecholamines in preventing the syrosingopine-induced amnesia. In this experiment, DA or NE was administered at one of 3 intervals after avoidance training. Since the catecholamines were given after training, the ef-

fects of these compounds of memory processes, as opposed to sensory-motor factors associated with acquiring the task, can be more directly assessed.

METHOD

Animals and Apparatus

Animals and apparatus are as described in Experiment 1.

Procedure

Mice were given 2.5 mg/kg syrosingopine 2 hr before passive avoidance training. Immediately, 10 or 90 min following training, independent groups received either 50 mg/kg DA or 0.75 mg/kg NE. A control group was given the drug vehicle 2 hr before being exposed to the apparatus and being allowed the step-through response. This group, however, received no footshock (NFS). Immediately after, these animals were given 0.75 mg/kg NE.

RESULTS

The results are shown in Table 3. The Kruskal-Wallis non-parametric analysis of variance found a significant treatment effect ($p<0.001$). Post-hoc comparisons with Mann-Whitney U tests are presented in Table 3. The injection of DA or NE prevented the syrosingopine-induced amnesia when given up to 10 min but not 90 min following avoidance conditioning. The step-through latencies of animals injected with DA or NE, 0 or 10 min post training were not statistically different from vehicle injected controls. Animals that received the catecholamines 90 min following training, displayed the syrosingopine impairment of retention. Finally, the short step-through latencies of the NFS-NE group indicate that the amnesia-blocking effect of NE was not due to a punishing effect of the injection procedure.

TABLE 3

TIME-DEPENDENT EFFECT OF DA AND NE ON SYROSINGOPINE-INDUCED AMNESIA*

Treatment	N	Mdn STL	p vs Syro+V
Syro+DA 0	12	205	<0.05
Syro+DA 10	13	251	<0.01
Syro+DA 90	11	33	NS
Syro+NE 0	15	300	<0.01
Syro+NE 10	14	233	<0.01
Syro+NE 90	10	42	NS
Syro+V	10	54	—
NFS+NE	8	16	NS

*Mice were injected with syrosingopine (2.5 mg/kg) 120 min before PA. The animals received a second injection of DA (50 mg/kg), NE (0.75 mg/kg) or the drug vehicle (V) at either 0, 10 or 90 min following training.

DISCUSSION

The results of the first experiment demonstrate that syrosingopine, a peripherally active reserpine analogue, produces amnesia for a passive avoidance task in a time and dose-dependent manner. In this paradigm, 2.5, 4.0 or 6.0 mg/kg syrosingopine given 2 hr before training produced retention impairments. Administration of these dosages at other treatment-training intervals produced no amnesia. Similarly, 1.0 mg/kg was without effect at any interval.

In a previous publication [20] we reported that 2.5 or 4.0 mg/kg reserpine produced amnesia if given 2–5 hr before avoidance training. The structural similarity and the correspondence between the time- and dose-effects of reserpine and syrosingopine on retention could suggest similar mechanisms of action in the production of amnesia. This mechanism could be via the depletion of peripheral catecholamines.

The results of the second and third experiments also support a peripheral interpretation of the syrosingopine-induced amnesia. Systemic administration of 0.5 or 0.75 mg/kg NE or 50 mg/kg DA given up to 10 min following training reversed the syrosingopine effect. Since these amines do not cross the blood brain barrier, the protection they afford against syrosingopine implies a role for peripheral catecholamines in the behavioral phenomena produced by this drug.

The role of peripheral mechanisms in learning and memory has not been well defined, although several models have been considered. For example, DiGiusto and King [3] suggested that sympathetic changes (i.e., increased peripheral catecholamines) induced by an aversive US can become conditioned to salient environmental stimuli and thus provide an informational cue regarding the necessity of an instrumental response. Cook and his associates [2] suggested that epinephrine or NE could serve as conditioned stimuli in an avoidance learning task, and finally, Gold and Van Buskirk [5, 6, 7] theorized that the hormonal consequence of an aversive stimulus promotes the storage of recently acquired information by facilitating memory storage processes.

It would seem that the rauwolfia alkaloids, reserpine and syrosingopine, could produce amnesia by interfering with such catecholamine-dependent peripheral memory modulating processes.

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