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Time-Dependent Effects of Reserpine on Retention of Passive Avoidance

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WALSH, T. J. AND T. PALFAI. *Time-dependent effects of reserpine on retention of passive avoidance.* PHARMAC. BIOCHEM. BEHAV. 8(2) 103–105, 1978. – Reserpine produced amnesia for a one-trial passive avoidance task when given 2, 3, 4, 5 hr before but not when given 1, 8, 12, 24 hr or 30 min before, or immediately, 90 min or 2, 4, 5, 6, 8, 9, 12, 24 hr following training. The results were discussed in terms of the reserpine effect on biogenic amines and their possible role in memory formation.

Amnesia Reserpine Memory Biogenic amines

RESERPINE, a drug that among several of its pharmacological effects, depletes both catechol and indole amines [2, 3, 6, 14, 18], was reported to produce retrograde amnesia (RA) in a variety of paradigms [1, 4, 8, 10, 15, 17, 20]. In the studies of Rake and his colleagues [1, 4, 15], the drug treatment resulted in RA for either passive or active avoidance training when given immediately but not 24 hr following conditioning. Similar findings were published by Kurtz and Palfai [10]. The latter authors used a discriminated escape situation and found that reserpine impaired retention when given immediately but not 90 min following the training session.

Contrary to these results, an unusual amnesic gradient was observed by Karpiak, Kirchmer and Rapport [8] following reserpine. Utilizing a passive avoidance situation, they reported that amnesia could only be observed if the drug was administered between 4 to 11 hr following training. The same dose given either 5, 60, 90, 120, 180 min or 17, 18 or 24 hr after training had no effect on subsequent retention. This finding is not only unlike the typical RA gradients frequently reported in the literature [1, 4, 10, 15], but raises some questions about potential mechanisms by which reserpine produces this phenomenon.

Since a number of publications implicate the biogenic amines, dopamine, norepinephrine and serotonin in both learning and memory [1, 3, 4, 9, 12, 16], it has been assumed, perhaps incorrectly, that the effects of reserpine on retention reflect its biochemical effects on these endogenous amines [1, 4, 8, 10, 15]. That is, perhaps the mechanism by which reserpine produces retention impairments is by altering the normal levels of biogenic amines during the early phases of memory formation. The Karpiak *et al.* data, on the other hand, imply that this amine-dependent phase occurs within a specific time interval several hours following the training trial.

To investigate the generality of the amnesic gradient reported by Karpiak *et al.* [8], we utilized the passive avoidance situation most frequently employed in studying RA [7] and administered an amnesic dose level of reserpine at different times before or after this training.

EXPERIMENT 1

The purpose of the first experiment was to establish a reserpine dose-response relation for amnesia in the passive avoidance paradigm used here. Since we reported [10,13] that administration of reserpine 2 hr prior to training produced amnesia reliably, this same interval was chosen to investigate the dose effect.

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 standard Econo plastic cages in temperature (70–72°F) and humidity (50–70%) controlled environment. 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 [7] was used. Briefly, the apparatus consisted of a Plexiglas covered V-shaped trough, which was partitioned 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 a.c. footshock

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TABLE 1

RETEST LATENCIES FOR GROUPS TREATED WITH VARIOUS RESERPINE DOSAGES 2 HR PRIOR TO PASSIVE AVOIDANCE TRAINING

Reserpine dosage	Median	N	p vs. 4.00 mg/kg
4.00	34	12	—
3.00	49	10	NS
2.50	13	13	NS
2.00	18	12	NS
1.50	35	13	NS
1.00	286	19	<0.01
0.50	221	13	<0.01

from a Grason Stadler Model 700 Constant Current Shock Generator.

Procedure

Ninety-two mice received either one of seven doses of reserpine (Serpasil, Ciba) intraperitoneally 120 min prior to passive avoidance training. 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 the 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

Group median step-through latencies are presented in Table 1, along with a group-by-group comparison via a series of post hoc Mann-Whitney U tests.

A Kruskal-Wallis nonparametric analysis of variance indicated that reserpine produced a significant dose-dependent treatment effect ($H(6) = 22.49$, $p<0.001$). As shown in Table 1, 1.5 mg/kg was the lowest dose still producing amnesia. Lower doses (1 and 0.5 mg/kg) appeared to have no effect on retention of this task..

EXPERIMENT 2

In this experiment, the time dependent effect of 2.5 mg/kg reserpeine on retention was investigated. This dose was chosen because it was the highest dose with relatively the smallest effects on initial step-through latencies. The purpose of the experiment was to investigate the generality of the unusual amnesic gradient reported by Karpiak *et al.* [8]..

METHOD

Animals

Mice of the same description as previously, were used.

Apparatus

The passive avoidance apparatus was the same as before.

TABLE 2

RETEST LATENCIES 7 DAYS AFTER PASSIVE AVOIDANCE TRAINING

Treatment	Median	N	p vs. DW cont
Res 24 hr pre	296	15	NS
Res 12 hr pre	290	13	NS
Res 8 hr pre	248	12	NS
Res 5 hr pre	44	19	<0.01
Res 4 hr pre	19	24	<0.01
Res 3 hr pre	59	20	<0.01
Res 2 hr pre	46	40	<0.01
Res 1 hr pre	198	28	NS
Res 30 min pre	296	20	NS
Res immed post	280	20	NS
Res 90 min post	300	20	NS
Res 2 hr post	300	15	NS
Res 4 hr post	300	15	NS
Res 5 hr post	300	18	NS
Res 6 hr post	300	15	NS
Res 8 hr post	300	12	NS
Res 9 hr post	300	14	NS
Res 12 hr post	300	15	NS
Res 24 hr post	300	17	NS
DW 2 hr pre	300	8	—

Procedure

One hundred-ninety-one mice received 2.5 mg/kg reserpine (Serpasil, Ciba) intraperitoneally at one of 9 time intervals prior to passive avoidance training. These intervals were 24, 12, 8, 5, 4, 3, 2 or 1 hr or 30 min preceeding passive avoidance training. One hundred-seventy-one animals received the reserpine injection at one of 10 intervals following training. The post training intervals were immediately, 90 min 2, 4, 5, 6, 8, 9, 12, or 24 hr following the avoidance training. Eight animals received distilled water (drug vehicle) 2 hr before training.

RESULTS

In all cases, the reserpine injections resulted in characteristic symptoms, which included ptosis, diarrhea and hypokinesia. The onset of these symptoms typically began between 30 and 60 min following injection and persisted for approximately 36–48 hr. At the time of retest, no overt behavioral symptoms were evident, however, The overall mortality rate for all reserpine treatment groups was 12%.

Group median retest latencies are presented in Table 2, along with a group by group comparison via a series of post hoc Mann-Whitney U tests.

A Kruskal-Wallis nonparametric analysis of variance indicated that reserpine did produce a significant time-dependent treatment effect, $H(18) = 44.81$, $p< 0.001$.

As shown in Table 2, reserpine produced retention impairments only if administered 2, 3, 4 or 5 hr prior to passive avoidance training. Groups treated with reserpine at other intervals were not significantly different from the vehicle-injected controls (DW).

DISCUSSION

The results of this experiment demonstrate a time-dependent reserpine effect on retention of a single passive

avoidance task. In this paradigm, the drug produced amnesia only if it was given 2 to 5 hr before training. No amnesia was seen when the administration occurred either earlier, nearer to training or any time after conditioning. Although these findings are somewhat different from those of others [1, 4, 10, 15] the difference might be attributed to procedural variations among these experiments. The procedure used here was similar to that of Karpiak *et al.* [8], however, but we found no evidence for an inverted U-shaped time-dependent effect from reserpine on retention. The generality of this phenomenon, therefore, was not supported.

While the present experiment is procedurally simple, multiple explanations might be offered for these results. For example, it is possible that reserpine might have impaired sensory and/or motivational processes necessary for the acquisition of the task. Although several investigators [5, 11, 19] have suggested that alterations of biogenic amines might result in modified sensitivity to footshock, we found that in this situation this was not the case [13]. An alternative that might be suggested is that the apparent anterograde amnesia induced by reserpine could be viewed as a case for state-dependent learning. Similar to the findings of Kurtz and Palfai [10], however, we found that reserpine did not produce state-dependent learning in the same behavioral task as used in this study [13].

Finally, it might be suggested that the reserpine injection might have altered neurobiological mechanisms critically involved in the formation of long-term memory. In this context, it is of particular interest that the anterograde amnesic gradient in this study parallels the time course of the drug's effects on biogenic amines following a similar dose [2]. However, depletion of brain biogenic amines and a drug's time-dependent amnesic effects may or may not be related.

REFERENCES

1. Allen, C., B. S. Allen and A. V. Rake. Pharmacological distinctions between "active" and "passive" avoidance memory formation as shown by manipulation of biogenic amine active compounds. *Psychopharmacologia* 34: 1–10, 1974.
2. Azzaro, A. J., G. R. Wenger, C. R. Craig and R. E. Stitzel. Reserpine-induced alterations in brain amines and their relationship to changes in the incidence of minimal electroshock seizures in mice. *J. Pharmac. exp. Ther.* 180: 558–568, 1972.
3. Carlsson, A., E. Rosengren, A. Bertler and J. Nilsson. Effect of reserpine on the metabolism of catechol amines. In: *Psychotropic Drugs*, edited by S. Garattini and V. Ghetti. Amsterdam: Elsevier, 1957, pp. 363–372.
4. Dismukes, R. K. and A. V. Rake. Involvement of biogenic amines in memory formation. *Psychopharmacologia* 23: 17–25, 1972.
5. Harvey, J. A. and C. E. Lints. Lesions in the medial forebrain bundle: Relationship between pain sensitivity and telencephalic content of serotonin. *J. comp. physiol. Psychol.* 74: 28–36, 1971.
6. Holtzbauer, M. and M. Vogt. Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.* 1: 8–11, 1956.
7. Jarvik, M. E. and R. Kopp. An improved one-trial passive avoidance learning situation. *Psychol. Rep.* 21: 221–224, 1967.
8. Karpiak, S. E., M. Kirchmer and M. Rapport. Reserpine inhibition of passive avoidance behavior: Time and dose responses. *Res. Communs Psychol. Psychiat. Behav.* 1: 149–154, 1976.
9. Kety, S. S. The biogenic amines in the central nervous system: Their possible roles in arousal, emotion and learning. In: *The Neurosciences: Second Study Program*, edited by F. O. Schmitt. New York: Rockefeller University Press, 1970, pp. 324–336.
10. Kurtz, P. and T. Palfai. Effects of reserpine on retention of escape reversal in mice: Absence of state-dependent learning. *J. comp. physiol. Psychol.* 11: 393–406, 1977.
11. Lints, C. E. and J. A. Harvey. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. *J. comp. physiol. Psychol.* 67: 23–31, 1969.
12. Oliverio, A. Neurohumoral systems and learning. In: *Psychopharmacology – A review of Progress*, edited by D. H. Efrom. Public Health Service Publication No. 1836, 1968.
13. Palfai, T., T. J. Walsh, B. J. Albala and O. M. Brown. Effects of l–D–hydroxyphenylalanine (L–Dopa) and d, l,5–hydroxytryptophan (d, l, 5–HTP) on reserpine-induced amnesia. *Psychopharmacology* 53: 269–276, 1977.
14. Pletscher, A., P. A. Shore and B. B. Brodie. Serotonin as a mediator of reserpine action in the brain. *J. Pharmac. exp. Ther.* 116: 84–89, 1956.
15. Rake, A. V. Involvement of biogenic amines in memory formation: The central nervous system indole amine involvement. *Psychopharmacologia* 29: 91–100, 1973.
16. Randt, C. T., D. Quartermain, M. Goldstein and B. Anagnoste. Norepinephrine biosynthesis inhibition: Effects on memory in mice. *Science* 172: 498–499, 1971.
17. Seiden, L. S. and D. D. Peterson. Blockade of l–dopa reversal of reserpine-induced conditioned avoidance response suppression by disulfiram. *J. Pharmac. exp. Ther.* 163: 84–90, 1968.
18. Shore, P. A. Reserpine: A survey of its pharmacology. In: *Handbook of Neurochemistry*, Vol. 6: *Alterations of Chemical Equilibrium in the Nervous System*, edited by A. Lajtha. New York: Plenum, 1971, pp. 349–356.
19. Tenen, S. S. The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. *Psychopharmacologia* 10: 204–219, 1967.
20. Weiskrantz, L. and W. A. Wilson, Jr. Effects of reserpine on learning and performance. *Science* 123: 1110–1118, 1956.