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BRIEF COMMUNICATION

Comparison in Mice of the Amnestic Effects of Cycloheximide and 6-Hydroxydopamine in a One-Trial Passive Avoidance Task [1]

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RAINBOW, T. C., J. E. ADLER AND L. B. FLEXNER. *Comparison in mice of the amnestic effects of cycloheximide and 6-hydroxydopamine in a one-trial passive avoidance task.* PHARMAC. BIOCHEM. BEHAV. 4(3) 347–349, 1976. – To test further the hypothesis that cycloheximide (CXM)-induced amnesia is due in part to its effects on the central adrenergic system, a comparison was made in mice of the effects of the antibiotic and of 6-hydroxydopamine (6-OHDA) on memory of a one-trial passive avoidance task. Both drugs produced amnesia 24 hr after training but unlike CXM, 6-OHDA had no effect on memory 20 min after training.

Cycloheximide 6-Hydroxydopamine Amnesia

TRAINING in the presence of cycloheximide (CXM), an inhibitor of protein synthesis, results in loss of memory in several training situations [7, 11, 17]. While the amnestic effects of CXM are often attributed solely to inhibition of protein synthesis, there is increasing evidence that it has side effects on the adrenergic system and that these side effects may contribute importantly to the amnesia. Thus the loss of memory induced by the antibiotic and its analog, acetoxycycloheximide, is prevented by treatment with adrenergic stimulants [3,16] or monoamine oxidase inhibitors [4]. Both antibiotics cause a loss of tyrosine hydroxylase activity [6,18], both decrease the rate of synthesis of norepinephrine (NE) and dopamine (DA), and both cause an increase in levels of NE and DA (in spite of a depressed rate of synthesis) suggesting additional modifications of catecholamine (CA) metabolism [5,8].

The severe, chronic central adrenergic denervation caused by 6-hydroxydopamine (6-OHDA) offers an opportunity to evaluate directly the role of the adrenergic system in memory. If CXM affects only the adrenergic system one might anticipate that its effects would be reproduced in animals treated with 6-OHDA. We have found that, like CXM, the chronic central denervation produced by 6-OHDA results in amnesia for a one-trial passive avoidance response at 24 hr. Unlike CXM, however, 6-OHDA has no significant effect on memory shortly after training.

METHOD

Male C57b1/6J mice were housed 4 to a cage in a soundproof room at 24.5° artifically lighted from 7 a.m. to 7 p.m. Food and water were ad lib.

Under Nembutal anesthesia (75 mg/kg), 25 μg of 6-OHDA HBr (Regis) in 5 μl of saline containing 0.1% ascorbic acid was injected into each lateral ventricle. Control mice were injected in the same way with ascorbic acid vehicle. Mice were then isolated for at least 2 weeks before being used for behavioral or biochemical studies. By the end of the first week, cage behavior, feeding and drinking appeared normal. Postoperative mortality of mice treated with 6-OHDA was 10%. CXM (120 mg/kg) was injected subcutaneously 30 min before training.

For the CA assays on the cerebral hemispheres, mice were killed by cervical dislocation. CAs were extracted and NE assayed according to the method of Anton and Sayre [2] as modified by Moore and Smith [10]. DA was assayed as described by Adler [1].

The two-compartment passive avoidance box was identical to that described by Randt *et al.* [14]. Mice were placed in the small compartment of the box 40 sec before the door was opened to the large compartment. After entering, the door was closed and 5 sec later a 0.16 mA scrambled foot shock lasting 2 sec was automatically given. Mice were removed immediately to their home cages and

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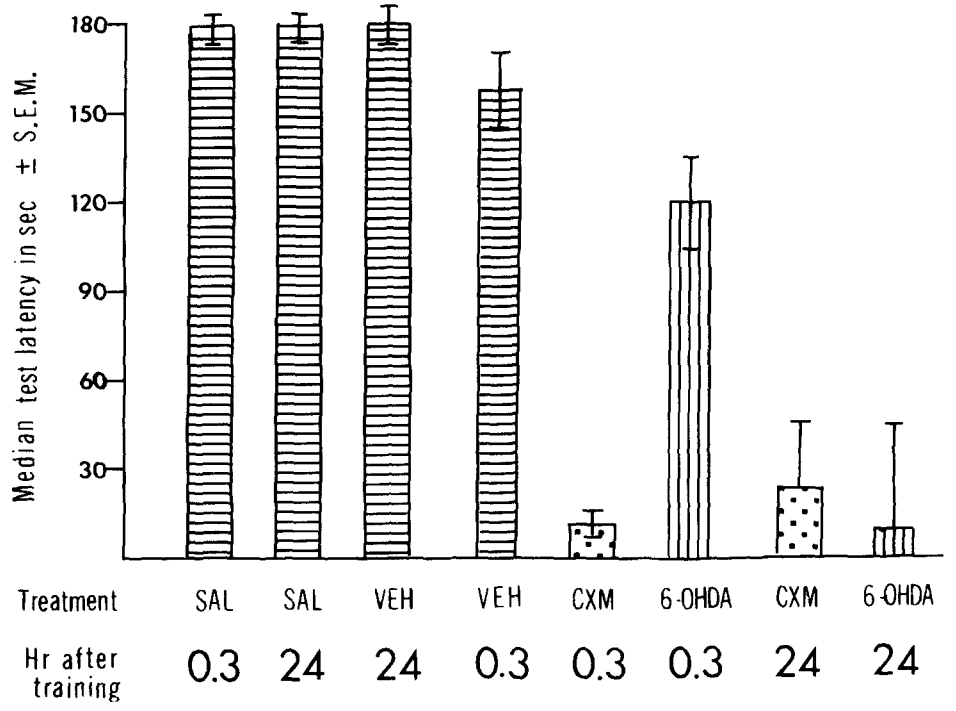

FIG. 1. Effects of treatment with CXM and 6-OHDA on memory of one-trial passive avoidance task 20 min and 24 hr after training. P values (Mann-Whitney U-test, 1-tailed): Saline 24 hr (n = 9) vs vehicle 24 hr (n = 9) 0.1; CXM 0.3 hr (n = 4) vs saline 0.3 hr (n = 5) = 0.008; 6-OHDA 0.3 hr (n = 5) vs vehicle 0.3 hr (n = 5) = 0.1; CXM 24 hr (n = 9) vs saline 24 hr = 0.001; 6-OHDA 24 hr (n = 9) vs vehicle 24 hr = 0.001. S.E.M. = standard error of the median.

tested for retention 20 min or 24 hr later. Latency to enter was taken as the time between orientation towards the large compartment and the time when all paws were on the bars of the large compartment. A maximum interval of 30 sec was allowed for orientation. Mice that failed to enter the large compartment within 3 min were removed and given a score of 3 min.

RESULTS

(a) Biochemical

The means ± SEM for cerebral NE and DA of the controls (n = 7) were 0.29 ± 0.02 and 1.06 ± 0.07 μg/g, respectively, uncorrected for 70% recovery. The means ± SEM for NE and DA after treatment with 6-OHDA one day after the behavioral experiments were, respectively, 0.05 ± 0.03 and 0.31 ± 0.05 μg/g. Thus 6-OHDA reduced the average level of NE by 83% and that of DA by 71%.

(b) Behavioral

As shown in Fig. 1, CXM produced marked amnesia both at 20 min (n = 4) and 24 hr (n = 9) after training. By contrast, treatment with 6-OHDA had no significant effect on memory at 20 min (n = 5) but, like CXM, caused severe amnesia at 24 hr (n = 9).

DISCUSSION

As stated above, there is evidence that can be interpreted to mean that CXM-induced amnesia results in part from interference with the central adrenergic system. To test this possibility further, we have compared CXM-induced amnesia of a one-trial passive avoidance task to that observed in mice that had a drastic loss of CAs after treatment with 6-OHDA. The two amnesias were both severe at 24 hr. Twenty minutes after training, however, those mice treated with 6-OHDA had a high level of memory; those with CXM, profound amnesia as also observed by Quartermain and McEwen [12] and shown not to be due to effects on activity [9]. Retention of memory at 20 min with 6-OHDA indicates normal acquisition; amnesia at 24 hr suggests a failure of further consolidation or a failure of retrieval. That failure of retrieval rather than of acquisition does occur with CXM has been shown by recovery of memory following treatment with monoamine oxidase inhibitors [4] or noncontingent shocks [11].

The meaning of the difference between the two groups at 20 min after training is obscure. Compatible with our results are those of Randt *et al.* [15] and Zornetzer *et al.* [19] who found retention of memory of a one-trial passive avoidance task for a short time after training that was conducted in the presence of an inhibitor of DA-βhydroxylase or of tyrosine hydroxylase. The early amnesia after CXM suggests that its effects on the central nervous system differ from those of 6-OHDA and the inhibitors of CA synthesis. This difference may involve only the adrenergic system or may be due to interference with other aspects of neuronal function.

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