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BRIEF COMMUNICATION

DDC-Induced Retrograde Amnesias Prevented by Injections of dl-DOPS[1]

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HAMBURG, M. D. AND A. KERR. *DDC-induced retrograde amnesias prevented by injections of dl-DOPS.* PHARMAC. BIOCHEM. BEHAV. 5(4) 499–501, 1976. – Injection of a dopamine beta-hydroxylase inhibitor, diethyldithiocarbamate (DDC) in rats 30 min prior to training of a step-down passive avoidance task impaired performance of the task 24 hr later. Similarly, injection of DDC 30 min prior to testing blocked retrieval of a passive avoidance habit trained in normal rats the previous day. Injection of a direct norepinephrine (NE) precursor, dl-threo 3,4,-dihydroxyphenylserine (DOPS) 60 min before DDC prevented both amnesias. These data support the hypothesis that reduced levels of NE are responsible for DDC-induced amnesias.

Memory Learning Retrograde Amnesia DOPS Antiadrenergic Norepinepherine

INJECTION of diethyldithiocarbamate (DDC), a dopamine beta hydroxylase (DBH) inhibitor in rats and mice have produced a retrograde amnesia of trained passive avoidance responses [3, 7, 15, 23]. When DDC injection preceded training of a step-down passive avoidance response by 30 min and testing occured within the first 6 hr after training, adequate memory retrieval was possible and the animal performed the trained response, but at longer training to testing intervals (1, 3, 5 or 7 days) a significant performance decrement was observed [16].

When DDC injection preceded testing rather than training again an amnesia was produced [16]. This amnesia was similar to that observed following treatment with diisopropyl florophosphare (DFP) [9, 11, 28] or physostigmine [2, 10, 14, 16], but unlike the anticolinesterases, the degree of amnesia produced by DDC was not dependent upon the age of the memory at the time of injection. When rats were provided a recall experience prior to either DDC or physostigmine treatment, the normally observed amnesia was prevented [15,16].

The copper chelating properties of DDC should produce a variety of influences on brain function. That the observed amnesias were due to the influence of DDC on norepinephrine (NE) was first supported by Cohen and Hamburg [7] who reported similar storage and retrieval amnesias following treatment with the beta-adrenergic receptor blocker, propranolol. In addition, Richardson *et al.* [24] have reported that daily injections of propranolol clearly disrupt DRL performance (differential reinforcement of low rates) for food reward. Also, memory formation of a shuttle avoidance task in mice was prevented by injection of another beta-adrenergic blocker dichloroisoproterenol (DCI) [12]. As further confirmation of the mechanism underlying the DDC-induced amnesias, we attempted in this study to prevent a DDC amnesia by pretreatment with a direct NE precursor, dl-theo 3, 4,-dihydroxyphenylserine (DOPS) which is converted to NE by the general aromatic amino acid decarboxylase and does not require DBH.

Although there are some reports of little increase in brain NE caused by the injection of DOPS [1, 13, 21, 22], following NE depletion either by treatment with reserpine, alpha methyl-p-tyrosine (AMT) or bis-(4-methyl-1-homopiparazinyl-thiocarbonyl-disulfide) (FLA-63), NE levels were significantly restored by DOPS [5, 8, 26, 27]. In addition, injection of DOPS has been shown to prevent known physiological effects of AMT and DDC on ovarian hypertrophy following hemiovariectomy [20] and progesterone induced stimulation of gonadotropin release [18].

METHOD

The experimental method employed in this study was indentical to that described by Hamburg and Cohen [7,15].

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Male albino rats (Sprague-Dawley, Holtzman strain) (250–350 g) were used. All animals were 2–3 months old upon arrival in the laboratory and were trained 7–21 days later. Rats were housed in community cages and had access to ample food and water at all times except when they were in the experimental room.

The task chosen was a step-down passive avoidance task [4, 6, 17, 19] in which the animals were taught to remain on a small raised platform in order to avoid foot shock. Length of time on the platform (step-down latency) served as a measure of task retention. The apparatus and procedure have been described elsewhere in detail [16]. Briefly, training consisted of 5 trials. On each trial the rat was placed on the platform and the step-down latency recorded. Animals were placed in a holding cage adjoining the experimental box for a 1 min rest period between each trial. On Trials 1–3, no foot shock was applied. On Trial 4, foot shock (approximately 0.4 mA) was administered for 15 sec. Trial 5 was conducted in a similar manner to Trial 4. On this trial, if the rat did not step down within 30 sec it was removed to the home cage and a step-down latency of 30 was recorded. On Trials 1–4, all animals stepped down within 30 sec (Median step-down latency for Trial 4 was 2.3 sec.

On the testing day, each animal was returned to the experimental room and placed on the platform for 1 trial. Upon step down or after 30 sec on the platform the experiment was terminated.

Drug injection occurred at different times relative to training and testing as indicated in the group procedures below. Each animal received either a subcutaneous injection of DDC (250 mg/kg; 2 ml/kg) or a subcutaneous injection of DOPS (200 mg/kg; 2 ml/kg) or both.

Forty-seven rats were used in this study. Animals in Group 1 (n = 10) were trained on Day 1 and tested the following day. Thirty min prior to testing they were injected with DDC. The procedure for Group 2 (n = 8) was similar to Group 1 except on the testing day each animal received an injection of DOPS 60 min prior to DDC treatment. Animals in Group 3 (n = 11) received DDC injection 30 min prior to training rather than prior to testing which occurred on the following day. The procedure for Group 4 (n = 10) was similar to Group 3 except DOPS treatment preceded DDC by 60 min. Finally, the procedure for Group 5 (n = 8) was similar to Group 2 with DOPS and DDC injections preceding testing; however, these animals received 5 training trials on Day 1, but no foot shock was administered. Table 1 summarizes the procedures for all groups.

RESULTS AND DISCUSSION

The mean and median step-down latencies for trials 4 and 5 of training and the testing trial were calculated for all groups and appear in Table 1. The scores of each group were compared with all other groups by Mann-Whitney U test to determine if the differences were significant. Two tailed p values were calculated by Siegel's method for direct critical values for Mann Whitney U test [25].

Significant impairment of the trained passive avoidance response occured when DDC was administered before testing ($p<0.01$ when compared to trial 5 of training). The short testing scores of Group 5 which received the same injection sequence as Group 2 but no foot shock during training indicated that the injections alone could not account for the lengthened step-down latencies of Group 2.

In Group 3, injection of DDC 30 min prior to training had no effect on the ability of the animals to learn the passive avoidance task as had been previously noted [16]. However, considerable amnesia was evident 24 hr later. When DOPS was administered 60 min before DDC injection (Group 4) no amnesia was observed on the following day (Group 3 testing scores significantly different from Group 4; $p<0.01$).

Our results indicate that: (1) injection of DOPS 60 min prior to DDC treatment prevents the normally observed DDC retrieval amnesia of a 1 day old passive avoidance response; and (2) injection of DOPS 60 min prior to DDC treatment will also prevent a DDC-induced amnesia of the same task. These data support the hypothesis that the memory deficits seen in animals following injections of DDC was consequent to changes in CNS levels of NE and not a result of other indirect actions of the drug.

TABLE 1

MEAN, MEDIAN AND STANDARD DEVIATION OF STEP-DOWN LATIENCIES IN SEC

	Group	N	Training Trial 4 mean	Training Trial 4 mdn	Training Trial 5 mean	Training Trial 5 mdn	Testing Trial mean	Testing Trial mdn
1.	Tr—1d—DDC 30m—Ts	10	2.64	2.80	28.98	30.00	8.23	3.76
			(0.97)*		(2.17)		(9.50)	
2.	Tr 1d DS 1h DDC 30m Ts	8	1.65	1.41	29.80	30.00	26.87	30.00
			(0.76)		(0.56)		(6.14)	
3.	DDC 30m Tr—1d— Ts	11	2.78	2.50	29.96	30.00	8.32	3.21
			(1.22)		(0.12)		(9.05)	
4.	DS 1h DDC 30m Tr—1d— Ts	10	2.14	1.99	28.88	30.00	23.23	25.78
			(0.90)		(3.13)		(8.81)	
5.	Tr_{NS} 1d DS 1h DDC 30m Ts	8	1.82	1.80	1.81	1.88	3.12	2.74
			(0.57)		(0.36)		(1.30)	

*Figures in parenthesis are standard deviations.

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