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BRIEF COMMUNICATION

Inhibition of Catecholamine Synthesis and Conditioned Avoidance Acquisition[1]

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AHLENIUS, S. *Inhibition of catecholamine synthesis and conditioned avoidance acquisition.* PHARMAC. BIOCHEM. BEHAV. 1(3) 347–350, 1973.–The effects of α-methyl-*p*-tyrosine methylester hydrochloride (α-MT) (200 mg/kg s.c.) and bis-(4-methyl-l-homopiperazinylthiocarbonyl) disulfide (FLA-63) (20 mg/kg s.c.) on the acquisition of a conditioned avoidance response (CAR) were studied. Both α-MT and FLA-63 reduced brain noradrenaline about 50 per cent, 6 and 1 hr respectively, after the injections. α-MT also reduced brain dopamine about 40%. Neither treatment with α-MT nor FLA-63 prevented the learning of a CAR. The acquisition of the CAR was followed over 3 test sessions and the experiment was designed to control for state-dependent effects of the drugs. The injection of FLA-63, but not α-MT was found to result in state-dependent learning. The performance of the CAR was affected by α-MT- but not FLA-63-treatment. It is concluded that the reduction of brain catecholamines obtained by α-MT or FLA-63 does not interfere with the final acquisition of a CAR.

α-Methyl-*p*-tyrosine DA-β-hydroxylase inhibition Conditioned avoidance acquisition State-dependent learning

VARIOUS stimulant drugs including amphetamine have been shown to improve retention of learned responses in animal experiments. This has been shown when the drug is given immediately (within 30 min) before or after learning trials [see 15]. Furthermore, *d*-amphetamine has been shown to facilitate the retention of verbal material learned under influence of the drug [13]. Amphetamine is known to cause a stimulation of catecholamine receptors via release of newly synthesized amines [see 6]. If the effects of amphetamine on catecholamines, noradrenaline (NA) and/or dopamine (DA) are the basis for its facilitation on acquisition and learning it would be of interest to see, whether drugs that block catecholamine neurotransmission can retard or prevent learning. In a recent study, where the DA-β-hydroxylase inhibitor diethyldithiocarbamate (DDC) was used, an impaired performance of a passive avoidance response was shown [19]. It was suggested that NA is essential in the memory formation of this task.

The aim of the present investigation was to elucidate the specific role of the catecholamines, NA and/or DA in the acquisition of an active avoidance response. The experiment was performed on mice and the acquisition of a Conditioned Avoidance Response (CAR) was followed under the influence of inhibitors of catecholamine synthesis, α-methyl-*p*-tyrosine, which inhibits the hydroxylation of L-*p*-tyrosine to *L*-DOPA [16,21], and bis-(4-methyl-l-homopiperazinylthiocarbonyl) disulfide (FLA-63) [7] which inhibits the β-hydroxylation of DA to NA [9, 10, 23].

METHOD

Animals

Experimental animals were female albino NMRI mice (Anticimex, Stockholm), 25–30 g.

Apparatus

The animals were trained in a shuttle box with the internal dimensions 20 x 14 x 10 cm. The two parts of the chamber were divided by a wooden partition with a square passage, 3 x 3 cm. The grid floor was connected to a 360 V power supply. In order to minimize variation due to the animal itself a large internal resistance of 2.8 MOhm was

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incorporated in the circuit.

The conditioned stimulus (CS), the sound of a house buzzer, was presented. If the animals failed to cross the passage between the two compartments within 5 sec after CS presentation, the unconditioned stimulus (US), the shock, was introduced in addition to the CS. A maximum of 5 sec US/CS was allowed before a trial was terminated. Experimental sessions consisted of 20 trials randomly distributed over 15 min. The delivery of CS and US were manually operated. Constant environment was provided by a sound-proof, airconditioned enclosure. Observations of the animals were made through a one-way screen. A conditioned avoidance response (CAR) was scored if the animals crossed the midline within 5 sec after CS presentation, and an escape response, if the animal crossed within the ensuing 5 sec under US/CS presentation. If the mouse remained in the same half of the chamber for more than 10 sec after CS presentation an escape failure was recorded. Furthermore, the number of intertrial responses were recorded.

Drugs and Experimental Procedure

The methyl-ester hydrochloride of DL-α-methyl-*p*-tyrosine (α-MT) (H 44/68, Hässle Mölndal) dissolved in isotonic saline (20 ml/kg), was given subcutaneously in a dose of 200 mg/kg. Bis-(4-methyl-l-homopiperazinyl-thiocarbonyl) disulfide (FLA-63, Astra, Södertälje) was dissolved in a few drops of acetic acid, the final solution made up with 5.5% glucose and given by the same route in a dose of 20 mg/kg (20 ml/kg).

In the α-MT-experiment, the animals were divided into two groups, one group (n = 21) receiving α-MT (200 mg/kg s.c.) 6 hr before the test sessions, and the other group (n = 23) receiving the same volume of the vehicle at a corresponding time. Six hr after the injection of α-MT it might be expected that there is a marked reduction in the catecholamine synthesis [cf. 24]. After 2 test sessions separated by 7 days, each group was further divided into two subgroups, one subgroup (n = 11) receiving α-MT and the other (n = 10) receiving saline. The third and final test was made 7 days after the second. To equate the different groups with regard to total amount of drug injected, all animals that received saline before the sessions, during 1 and 2, were given α-MT (200 mg/kg s.c.) 24 hr after the two test sessions. The FLA-63 experiment was made in analogy with the α-MT experiment. Animals were tested 1 hr after FLA-63, at a time when the inhibition of DA-β-hydroxylase can be considered maximal [cf. 23].

No changes in the gross behaviour of the animals were seen and all animals gained in weight throughout the experiment (+ one week after the last test session). As the performance of saline-treated animals in the α-MT (n = 11) and FLA-63 (n = 9) experiments did not differ with respect to CAR performance (77.5% vs. 87.5%, respectively in the 3rd session), these groups were pooled for comparisons with the different drug treated groups.

Biochemistry

Female mice of the same strain (20–25 g) were used. The doses and the drug injection procedures were the same as described for the behavioural experiments above. Animals were sacrificed 6 hr after α-MT or 1 hr after FLA-63 and the whole brains with the exception of the olfactory bulbs were rapidly removed and catecholamines were analysed spectrophotofluorimetrically after cation exchange chromatography. The purification and separation of the amines was made on Dowex 50W-X4, 4.0 mm I.D. x 72 mm, sodium form, columns as outlined by Atack and Magnusson [4]. The NA assay was made according to the method of Bertler *et al.* [5], and the DA assay according to Carlsson and Waldeck [8] with modifications as outlined by Atack *et al.* [3].

RESULTS

α-MT Experiment

Behaviour. In the first test session the two groups receiving α-MT (200 mg/kg s.c.) or saline 6 hr before the sessions, did not differ with respect to CAR performance (30% vs. 35%), but they were statistically different in the second test (40% vs. 60%, $p<0.01$, Mann-Whitney U-test). When the two groups were further split at the third test session, both groups receiving α-MT were lower with respect to per cent CAR than either group receiving saline (Fig. 1a). There were no statistical differences with respect to the number of intertrial responses in the different groups.

Biochemistry. The α-MT-treatment (200 mg/kg s.c.) resulted in a significant reduction of both NA and DA in the brain 6 hr after the injection of α-MT (Table 1). The reduction of NA was about the same as the reduction of DA when expressed as per cent of controls (37 vs. 43%).

FLA-63 Experiment

Behaviour. The FLA-63 (20 mg/kg s.c.) treated animals did not differ from the saline treated controls with respect to avoidance responding 1 hr after the injection in any of the two first test sessions (45 vs. 35% and 55 vs. 60%, respectively). In the third and final test the two groups that continued on the same treatment were higher with respect to CAR performance than the two groups that were tested under changed conditions (Fig. 1b). The number of intertrial responses were lowered in the group receiving FLA-63 after two saline sessions, when compared with the number of intertrial responses in the two groups tested under the same drug conditions ($p<0.02$). As assessed by gross observations the FLA-63-treated as well as α-MT-treated animals were indistinguishable from saline-treated controls.

Biochemistry. The FLA-63-treatment (20 mg/kg s.c.) resulted in a significant reduction of brain NA (Table 1), 1 hr after the injection. DA was unchanged by this treatment. Brain NA values after α-MT did not differ from those obtained after FLA-63 (0.24 vs 0.22 μg/g), while brain DA was below the values obtained after treatment with FLA-63 (0.80 vs. 1.34 μg/g).

DISCUSSION

In the present experiments it has been shown that a 50% reduction of brain NA induced by FLA-63 (20 mg/kg s.c.) does not interfere with the acquisition of a CAR. The general pattern in the FLA-63 experiments is that usually obtained in studies on state-dependent learning, i.e. what is learned under drug state is best remembered under the same conditions and any change in the state of the organism between training and testing results in a decrement in performance. This phenomenon has been described in animals and in man [see 18]. The statistical treatment of the present data speaks in favour of an asymmetrical effect,

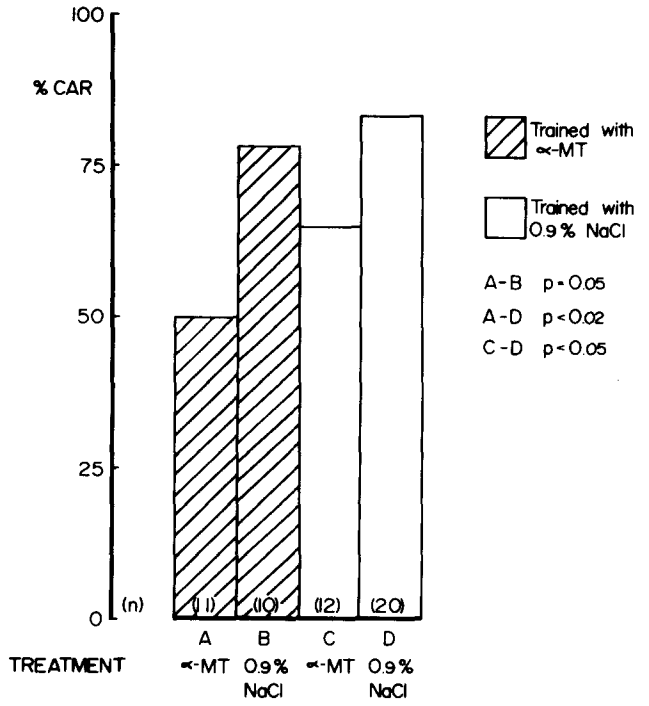

FIG. 1a. Effects of α-MT (200 mg/kg s.c.) on the acquisition of a CAR. The ordinate gives the median per cent CAR. In the first and second tests the animals were divided into 2 groups: α-MT (shaded bars) or saline (open bars). At the third test session, shown here, these groups were further divided into 4 subgroups. Statistical comparisons are based on the Mann-Whitney U-test after Kruskal-Wallis one-way analysis of variance [20].

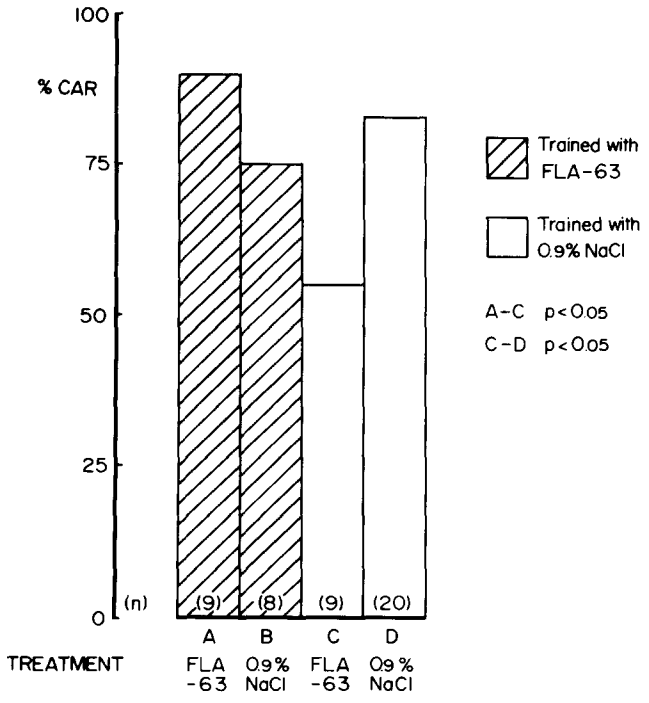

FIG. 1b. Effects of FLA-63 (20 mg/kg s.c.) on the acquisition of a CAR. For details see legend to Fig. 1a.

TABLE 1

EFFECTS OF α-MT AND FLA-63 ON BRAIN NORADRENALINE (NA) AND DOPAMINE (DA)

Treatment:	NA	DA
0.9% NaCl	0.42 ± 0.04 (n = 5)	1.26 ± 0.07 (5)
α-MT	0.24 ± 0.02 (5)*	0.80 ± 0.07 (5)*
FLA-63	0.22 ± 0.01 (5)*	1.34 ± 0.20 (5)†

*$p<0.001$ †N.S.$p>0.05$

The table gives the brain NA and DA values in μg/g ($\bar{X}$ ± S.D.). α-MT (200 mg/kg s.c.) was given 6 hr and FLA-63 (20 mg/kg s.c.) 1 hr before decapitation. Statistical comparisons are made with the control groups. Furthermore the α-MT- and FLA-63-treated animals did not differ with respect to NA ($p>0.05$), but with respect to DA ($p<0.001$). *t*-test after analysis of variance.

i.e. the performance deteriorated when the animals were changed from saline to drug but not vice versa. It cannot be excluded that the effects of DDC on passive avoidance reported by Randt *et al.* [19] were state-dependent.

An injection of α-MT (200 mg/kg s.c.) before the third test session gave a suppression of CAR whether the animals had received α-MT before the two previous test sessions or not. Animals given saline before the last test following two test sessions on α-MT showed an avoidance responding not different from controls receiving only saline (77.5 vs. 83.5% CAR). This shows that learning of a CAR is possible under the present α-MT treatment (Group B in Fig. 1a), but not performance of (cf. Groups A and C in Fig. 1a). A similar finding was obtained by Stolerman [22] who used a catecholamine receptor blocking agent, chlorpromazine: Treatment with chlorpromazine did not delay the learning of a positively reinforced behaviour as evidenced in a drug-free test session. The lever-press performance was depressed by chlorpromazine irrespective of whether the drug was given in the early test sessions or later during the acquisition of the task. A posttrial injection of reserpine has been shown to impair later performance of a CAR and this effect can be antagonized by L-DOPA, but not 5-HTP [11]. Impairment of a CAR was also found after a posttrial injection of DDC and it was concluded that NA may be important in the consolidation process. The present experiments, however, do not lend support to the idea that NA is critically involved in memory formation [cf. 11, 14, 17, 19], since learning and performance of a CAR was unaffected by the fairly marked depletion of brain NA induced by FLA-63 (cf. Groups A and B in Fig. 1b). The impairment of performance by FLA-63 in the third test after two tests on saline may be explained on the basis of state-dependent effects of the drug (Group C in Fig. 1b). That the unimpaired performance in Group A (Fig. 1b) is not due to tolerance to the effects of FLA-63 is shown by the effects of FLA-63 on the performance of the CAR in Group C, since these animals had received the same total amount of FLA-63 (see Methods).

It is possible that the decrement in the CAR acquisition after α-MT, in the present experiment, is due to interference with DA and not NA neurotransmission since the administration of FLA-63 did not prevent the performance of a CAR at a time, when brain NA levels were at the same

low level as after treatment with α-MT. Needless to say, identical reductions in whole brain NA levels do not implicate identical functional effects and further experiments are needed to clarify this issue.

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