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The Disruption of Conditioned Avoidance Response Following Selective Depletion of Brain Catechol Amines

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With 4 Figures in the Text

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It is generally accepted that the sedative action of reserpine is associated with a decrease in central monoamines, *i.e.* dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT), but opinions differ as to the relative role of these amines. In attempts to solve this problems reserpine-like drugs with somewhat selective actions on one of the amines have been used. However, the selectivity of drugs available so far has been insufficient to reach conclusive results (for review, see CARLSSON 1965).

It has been shown that α-methyltyrosine (α-MT) is a potent inhibitor of the tyrosine hydroxylase *in vitro* (NAGATSU et al. 1964) and *in vivo* (SPECTOR et al. 1965). Experiments in mice in the present laboratory have shown that this compound causes a selective depletion of brain catechol amines (CA), while leaving 5-HT essentially unaffected (CORRODI and HANSON, unpublished data).

It is a well known fact that the effect of reserpine on gross behaviour can be counteracted by L-3,4-dihydroxyphenylalanine (L-DOPA) (CARLSSON et al. 1957; BLASCHKO and CRUSCIEL 1960; SMITH and DEWS 1962). Conditioned reactions, especially the negatively reinforced conditioned avoidance response (CAR), are reduced by reserpine (ORSINGER 1961; HERZ 1960; OAKLEY 1963). This effect is partially antagonized by L-DOPA in mice and rats (SEIDEN and CARLSSON 1963, 1964); in cats the restoration of CAR is almost complete (SEIDEN and HANSON 1964). The DA level lowered by reserpine is concomitantly raised above normal values in all these species by L-DOPA.

This study was designed a) to investigate the effect of selective CA depletion, induced by the new mechanism described above, on the behaviour and CAR in cats and rats, b) to correlate behavioural data with the levels of 5-HT, NA and DA in brain and c) to study the effect of restoring CA levels by L-DOPA.

Methods

Behavioural

Nine mature cats, 4 males and 5 females, weighing 3.0—4.8 kg, were trained in a shuttle box with an electrified grid floor to 100% CAR. A detailed description of the box, the training procedure, the parameters and the criteria is given in an earlier paper (SEIDEN and HANSON 1964). The cats were in the shuttle box all day during the experiment, but were taken out at night and at intervals during the extraordinarily long experiments carried out in some cases. Each experiment started with a control trial, immediately followed by the injection of α-MT. A pilot study had shown 150—200 mg/kg of the compound to be effective on the CAR, the effect developing slowly during the first 12 hours and as a rule lasting for at least a day. Ten experiments were performed on 9 cats, 5 with 150 mg/kg and 5 with 200 mg/kg. In 4 cases the cats were returned to the box immediately after injection for testing during the following 8 to 10 hours. In 6 cases the tests were not started until 12 hours after the injection (Fig. 1). α-MT[1] was given i.p. in 10 ml 0.9% saline, pH 6.0.

In 7 experiments the cats were injected with L-DOPA, one (No. I) at 2 different experiments and one (No. V) 3 times in increasing doses during the same experiment. Cats who had received 150 and 200 mg/kg α-MT were given 75 and 100 mg/kg L-DOPA, respectively. The injections of L-DOPA were given during the maximum effect of α-MT, *i.e.* after about 15 hours, and further trials performed until the influence of L-DOPA, and eventually α-MT had passed, if the animal had not gone into an irreversible state and died. L-DOPA was dissolved in 25—30 ml 0.9% saline and administered i.p.

Cats No II, VI and IX were controlled regarding latency and CAR in temporary congruence with the earlier test sessions but with α-MT and L-DOPA exchanged for the same volumes of 0.9% saline with adjusted pH. These cats were chosen for control trials, because their performance, measured only as CAR disruption, was not conclusive (Fig. 2).

Three cats who seemed not to recover from the effect of α-MT were placed in a thermostat room at 27°C and were treated with s.c. and i.p. saline-glucose injections, but none was saved by these measures, nor by DOPA-injections.

Six male rats weighing 325—375 g of the Sprague-Dawley strain were trained to a CAR by lever pressing of at least 75% in the Skinner box (GRASON-STADLER) with the following schedule: conditioned stimulus (CS) red light for 10 sec; unconditioned stimulus (UCS) 1 mA electric shock

[1] I am indebted to AB Hässle through Dr. H. CORRODI for generous supplies of α-MT in the form of preparation H 44/68 (α-methyltyrosine methylester-HCl).

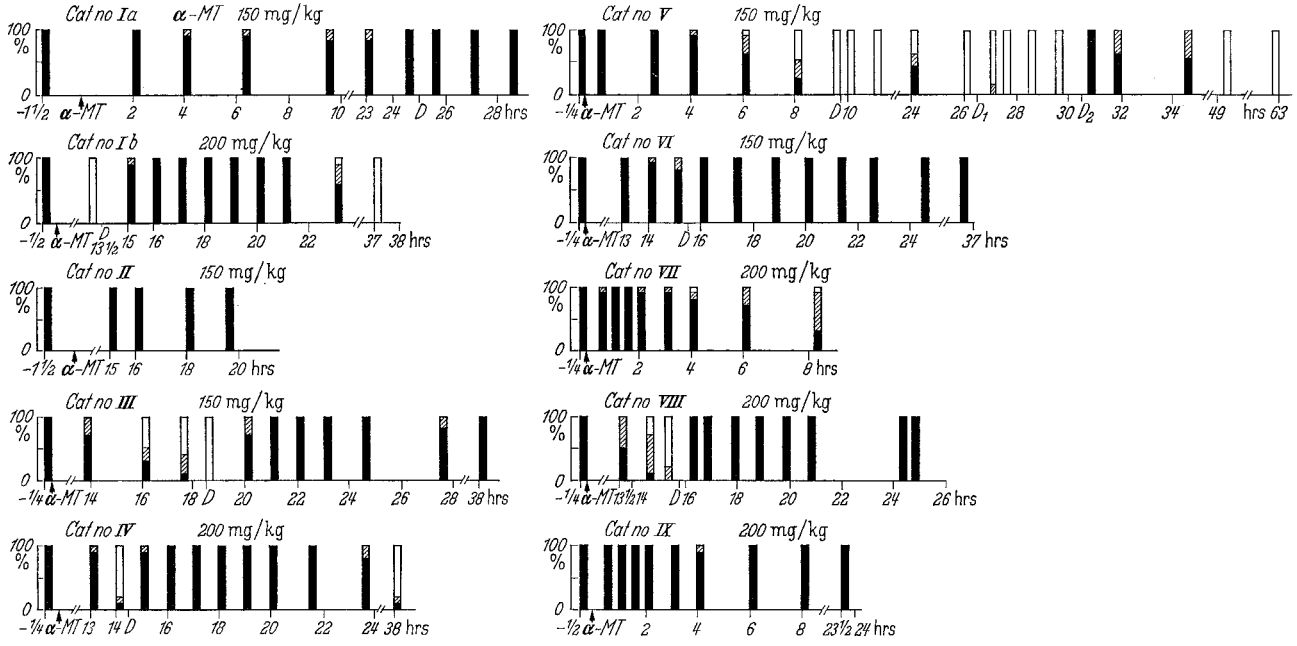

Fig. 1. Percent avoidance and escape per session of 10 CS at different times in 10 experiments with 9 cats treated with α-MT 150 and 200 mg/kg i.p., and in 6 cases with L-DOPA 75 and 100 mg/kg i.p. respectively. Cat No. V had L-DOPA 25, 50, and 75 mg/kg at D, D_1, and D_2. ■ avoidance responses (CAR); ▨ escape responses; □ failure to avoid or escape

of 1 sec duration through the feet; shock-shock interval 10 sec, response-shock interval 20 sec. The percentage avoidance was calculated from the number of CAR out of the total number of CS, which could be read on a counter as could the total number of lever-pressings. Lever-pressings without connection to CS or UCS were extremely rare. As a rule the training of the rats had been performed in periods of 4 hours with readings of the counter every $^1/_2$—1 hour. During the first $^1/_2$ hour gradual

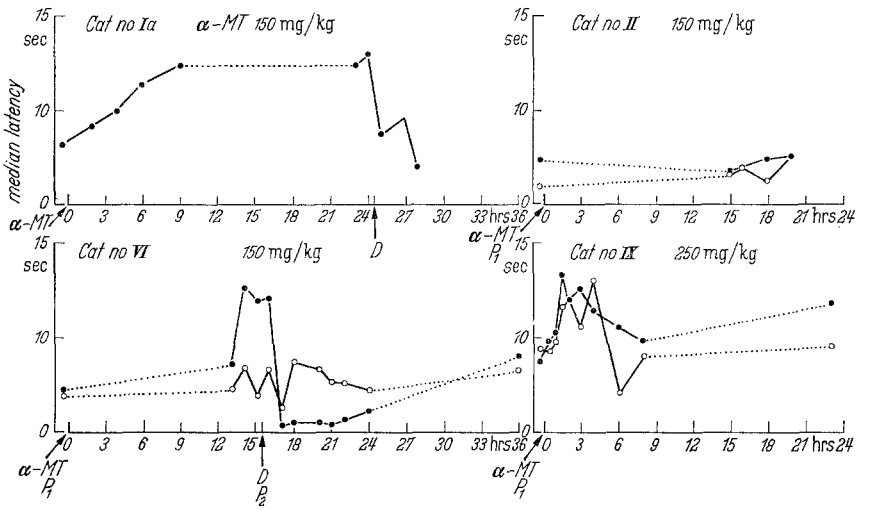

Fig. 2. Median latency in sec per session of 10 CS in 4 cats, where the suppression of CAR or the reversal by L-DOPA is not evident from Fig. 1. *D* L-DOPA 75 mg/kg; P_1 10 ml saline; P_2 30 ml saline. Black dots active substances, open dots placebo experiments

improvement was regularly observed, irrespective of the level of performance, the "warming up" period (Bernstein and Cancro 1962). This period could be more or less pronounced in different individuals but also for the same animal on different occasions. To eliminate its influence on the results to a certain degree, the percentage of CAR is plotted as the difference between performance under the influence of placebo (0.9% saline) and active substances (Fig. 3). For rats No. 54, 73 and 74 the timings of placebo and drug experiments were identical. For the rats No. 51, 52 and 57 a behavioural profile was calculated from a forgoing 5 hours placebo experiment and the last training period, when the rats had reached a constant level of at least 75% CAR (Fig. 3). In the placebo experiment 5 ml saline was injected i.p.; α-MT was given in 5 ml saline. Three rats (No. 54, 73, 74) were treated with 125 mg/kg, the remaining (No. 51, 52, 57) with 250 mg/kg. Rat No. 51 and 57 received 50 mg/kg L-DOPA (in 10 ml 0.9% saline) and L-DOPA-methylester-HCl (in 5 ml 0.9% saline) respectively in an i.p. injection 18 hours after α-MT.

Amine analysis

For this part of the study 23 cats and 12 rats were used. The amine levels of whole brain in untreated animals and at different times after the injection of α-MT and—in cats only—L-DOPA are evident from the Table. The rats were sacrificed by decapitation and the cats by exsanguination after nitrous oxide anesthesia. The amine levels were determined on whole brain and the analysis for 5-HT made according to the method of BERTLER (1961), for NA according to BERTLER, CARLSSON and ROSENGREN (1958), and DA according to CARLSSON and WALDECK (1958) with the modification described by CARLSSON and LINDQUIST (1962).

Results

Behavioural

In 8 out of 9 cats treated with α-MT 150—200 mg/kg i.p. a certain sedation of varying depth and duration was noted. In all cats marked miosis and a more or less pronounced photophobia were observed. In a few cases there was a single vomiting in connection with the injection. No other autonomic signs and no motor disturbances were seen on inspection. In two cats under strong influence of the compound the rectal temperature was measured to 38.0°C.

The individual reactions of the cats to the compound varied considerably. One cat treated with 150 mg/kg died (after a complete disruption of CAR), while another had unchanged latencies on CS, no escape and only slight sedation and eye symptoms. In fact 3 additional cats died 2—5 days after treatment with 200 mg/kg showing a picture of exhaustion, lying on side—but capable of walking without ataxia when handled—anorexia, dehydration and hypothermia. Of these cats 2 were males (4.0 and 4.2 kg) and 2 females (3.0 and 3.3 kg). The tissues of 3 of these cats (No. I, IV, VI) were analyzed for catechol amines and one (No. IV) also for 5-HT (Table).

In all but one (No. II) of the 10 experimental sessions the CAR decreased from the original 100% to between 0 and 80% except for No. IX, whose avoidance decreased to 90% and No. II who showed no change in CAR or mean latency after α-MT, compared with the placebo experiment (Fig. 1 and 2). In experiment No. I a) the compound had a slight but definite effect; there was overlapping between the lowest latencies after α-MT treatment and one single high latency value of the control trial before injection. In many experiments there was—in the course of a single trial—a successively increasing latency and eventually a disappearance of CAR followed by escape reactions, and in some cases no escape at all ("fatigue"). In this way often a single trial reflected in short the general course of the behavioural impairment after α-MT (see Fig. 1, No. III, 14 to 18 hours, No. IV, 13 to 14 hours, No. VIII, 13 to

15 hours). The prolonged latencies to CS seemed to depend more on a delayed initiation than to a lengthened running time (POSLUNS 1962); the movements remained quick and lissome. The spontaneous crossings of the hurdle were very few during the control session and even less under the influence of α-MT.

After the L-DOPA injection (7 experiments in 6 cats) there was in all 6 animals a reversal of the α-MT effect, with a shortening of latencies and

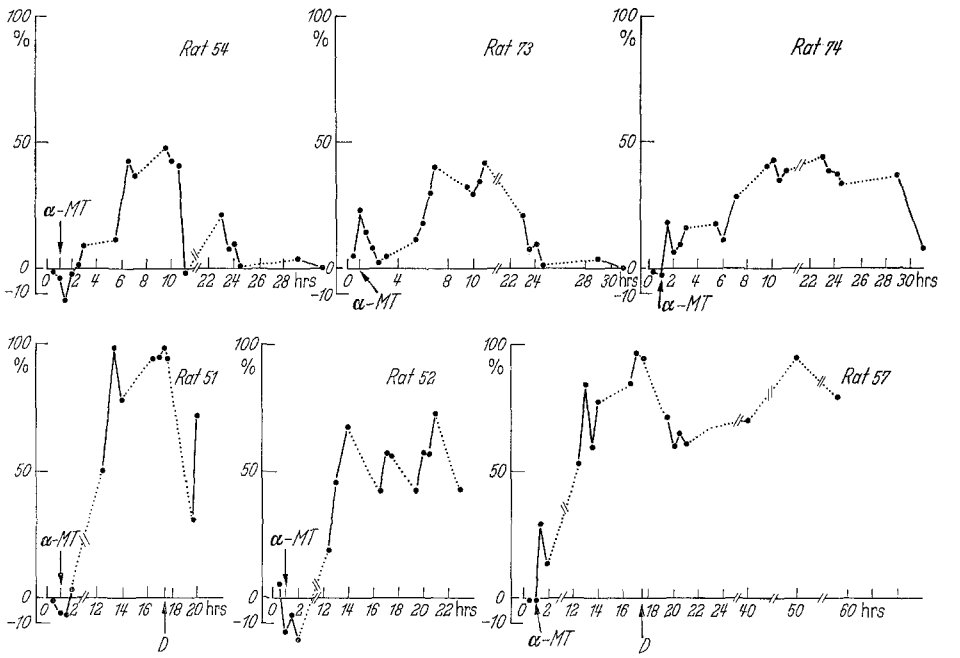

Fig. 3. Difference in percent CAR for placebo (5 ml saline) and α-MT-upper row 250 mg/kg, lower row 125 mg/kg. All injections i.p. and marked with an arrow. Second arrow in Rat 51 indicates injection of 50 mg/kg L-DOPA and in rat 57 50 mg/kg L-DOPA methyl ester-HCL. ——— registration; ------ no registration; ----//---- no registration, rat out of the test box

return of CAR in $^1/_2$—1 hour. The latency usually became much shorter than during the control trial, and the number of spontaneous crossings increased, but not to the extent of making judgements about the presence of a CAR difficult. The cats were excited and moved quickly, had piloerection and maximal mydriasis. The effect of L-DOPA lasted for 2—8 hours, and was in 5 cases followed by a return to persisting α-MT action, which in 3 cats terminated fatally within $2^1/_2$ to $5^1/_2$ days.

Cat No. V, who was the first to be tested with the α-MT and L-DOPA combination received only 25 mg/kg L-DOPA, to which was later added 50 and 100 mg/kg. The first and second doses caused no reversal, but the

Table. *5-HT, NA and DA levels in μg/g whole brain of the cat, after α-MT 150 mg/kg, L-DOPA 75 mg/kg*
(Letters read vertically refer to the same animals.) M ± SEM

	Untreated	15 hrs after α-MT	16 hrs after α-MT 1 hr after L-DOPA	19.5 hrs after α-MT 4.5 hrs after L-DOPA	Data of animals used for behavioural studies
5-HT	a) 0.319 b) 0.323 c) 0.312 0.318 ± 0.003	a) 0.209 b) 0.430 c) 0.283 d) 0.355 0.319 ± 0.047			b) 0.280
NA	d) 0.265 e) 0.143 f) 0.204 g) 0.204 h) 0.227 i) 0.272 j) 0.214 0.218 ± 0.016	a) 0.103 b) 0.208 c) 0.092 d) 0.210 0.153 ± 0.032	a) 0.206 b) 0.224 c) 0.300 0.243 ± 0.026	a) 0.247 b) 0.283 c) 0.177 0.236 ± 0.029	a) 0.026 (No. V, dead 3 days after α-MT 150 mg/kg) b) 0.014 (No. IV, dead 5.5 days after α-MT 200 mg/kg and 40 hours *after* DL-*DOPA 150 mg/kg*) c) 0.034 (No. I, dead 2.5 days after α-MT 200 mg/kg and 2 days after L-DOPA 100 mg/kg) 0.025 ± 0.002
	$t = 2.01$, $p > .05$				
DA	d) 0.335 e) 0.247 f) 0.276 g) 0.295 h) 0.290 i) 0.318 j) 0.301 0.295 ± 0.011	a) 0.056 b) 0.106 c) 0.075 d) 0.120 0.089 ± 0.015	a) 1.170 b) 1.684 c) 2.660 1.838 ± 0.440	a) 0.498 b) 0.741 c) 0.703 0.647 ± 0.076	a) 0.036 b) 0.041 c) 0.039 0.039 ± 0.002
	$t = 11.6$, $p < .001$				

third injection gave extremely short latencies and a complete return of CAR.

In rats the i.p. injection of α-MT, 125 mg/kg, caused a certain sedation with diminished spontaneous locomotion, slight miosis and ptosis and a mean fall of rectal temperature of 1°C normalized in 26 hours. After 250 mg/kg there were pronounced sedation, eye symptoms, and weight loss due to dehydration and anorexia. All 3 rats died 19 hours to about 2 days after the injection.

L-DOPA and L-DOPA-methylester-HCl caused within 30 min a striking but short reversal of the α-MT effect with excitement, increased motor activity, piloerection and protruding, mydriatic eyes.

All rats treated with α-MT in doses of 125 and 250 mg/kg demonstrated a decrease in CAR (Fig. 3). In the first group, which had received 125 mg/kg, the decrease was most pronounced between the 5th and 10th hour after injection. After 23 hours the CAR had returned to its normal level in two rats but was still low in one. The second group showed no definite change in CAR during the first hour after injection, but when returned to the test situation 12 hours later they soon reached a zero level of performance with no response to CS or UCS. In this state two of the rats received 50 mg/kg L-DOPA and L-DOPA-methylester-HCl respectively resulting in the rapid development of a brief evanescent increase in CAR. One animal died soon afterwards in the box and the other two were taken out of the experiment as they did not seem to recover from the α-MT effect. Both of them died later.

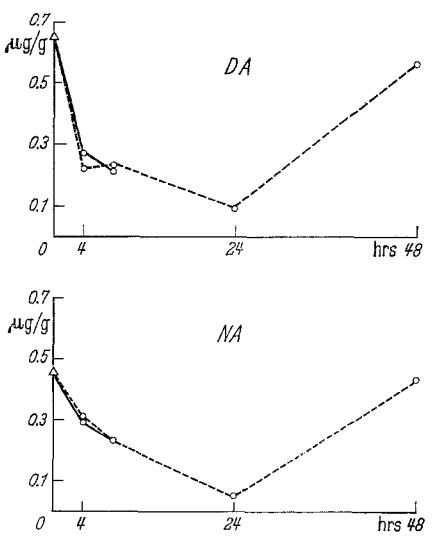

Fig. 4. DA and NA levels in µg/g whole brain in the rat after i.p. treatment with α-MT. ----- 250 mg/kg; ——— 125 mg/kg. Each symbol represents a single brain; △ normal average value

Chemical

Fifteen hours after the i.p. injection of 150 mg/kg of α-MT to cats no change in the 5-HT level of whole brain was observed (Table). Neither did cat No. IV, who died $5^1/_2$ days after the treatment with α-MT, show any change in 5-HT. In contrast the DA levels were reduced. The NA values were lower than in the controls, but the difference was not statistically significant. The addition of L-DOPA, 75 mg/kg, increased the DA

levels considerably within one hour. After another $3^1/_2$ hours DA had fallen to about one-third. The NA values were higher after the L-DOPA treatment, but the differences were not statistically significant. All 3 cats who died after the α-MT treatment and whose brains were analyzed had extremely low values of CA. The mean level of NA was 0.025 μg/g ($t = 6.89$, $p < .001$, compared to untreated animals), DA 0.039 μg/g ($t = 15.06$, $p < .001$). Also the amine levels of the heart, spleen and adrenals were very low in these cats (data to be published).

In the rats treated with α-MT the cerebral CA levels were significantly reduced (Fig. 4). As judged from the limited material the effect seems to develop in a few hours and last for at least 24 hours.

Discussion

The gross behaviour of the cats and rats treated with α-MT reminded much of that seen after reserpine treatment. Thus in both species sedation was noted, but was generally not as pronounced as after reserpine. Especially in the cats the speed of the movements was less affected than after reserpine treatment. In fact it was rather normal, even though the latencies were prolonged and the CAR disrupted. Both species demonstrated after α-MT treatment pronounced miosis. The cats showed marked photophobia but no relaxation of the nictitating membranes. In the rats ptosis was observed. The α-MT treated rats showed the typical hunched-back position and could hardly be distinguished from reserpine treated rats.

The time for development of the effects of α-MT and reserpine on CAR and gross behaviour was almost the same, but the duration of effect is longer after reserpine. However, α-MT was more difficult to dose, and 4 out of 9 cats and 3 out of 6 rats never recovered.

The "fatigue" phenomenon seen in the α-MT treated cats has been observed in similar experiments with reserpine.

As might be expected, the cats showed a more variable behaviour than the rats intra- as well as interindividually.

In both species there was a pronounced decrease of the CA, especially DA, which also in mice reaches a lower level than NA (Corrodi and Hanson, unpublished data). No effect on 5-HT has been observed in the species so far examined (cat and mouse, Corrodi and Hanson, unpublished data). The administration of L-DOPA to α-MT treated animals caused a rapid rise in DA and a slower and less pronounced but more sustained rise in NA. The cats, who participated in the behavioural experiment and died after the treatment with α-MT, had an almost complete depletion of CA in the brain, while the single value of 5-HT was quite normal.

It should be noted that both the behavioural and biochemical effects of α-MT are slow in onset and may, in fact coincide. However, most of the biochemical data given in the Table have been obtained from animals not stressed by the testing on CAR. Further investigation is needed to elucidate the possible role of stress for the biochemical effects of α-MT. The "fatigue" phenomenon mentioned above for α-MT suggests that such a stress influence exists. The L-DOPA-induced reversal seemed to be somewhat more prolonged after α-MT than after reserpine. This might be related to the remaining storage capacity of the specific intraneuronal monoamine granules after treatment with α-MT. It may be recalled that SMITH and DEWS (1962) found the action of DOPA to be shortened but intensified after pretreatment with reserpine.

It would appear from the present data that the selective CA depletion in brain induced by α-MT leads to disruption of CAR and that restoration of CA levels by L-DOPA results in restoration of CAR. The data thus support the view that CA neurons in brain play a role in CAR. Nothing conclusive can be said about the relative importance of DA and NA. In α-MT treated animals, whose intraneuronal storage granules are presumably intact, restoration of DA levels with L-DOPA must be assumed to restore the transmission mechanism of DA neurons. However, also the function of NA neurons may be restored, since part of the DA is converted to NA; furthermore DA may possibly take over some of the transmittor function of NA.

The fact that 25 and 50 mg/kg L-DOPA (cat No. V) did not cause return of CAR, though the DA level in the brain probably was normalized (cf. Table, high DA level after 75 mg/kg L-DOPA), speaks against DA beeing essential for CAR.

Summary

Selective depletion of catechol amines (CA) in brain—with little or no depletion of 5-hydroxytryptamine—was induced by α-methyl-tyrosine methylester-HCl. Concomitant disruption of conditioned avoidance responses (CAR) was observed in cats (Shuttle box) and rats (Skinner box). Restoration of the CA levels in the brain by L-DOPA resulted in a restoration of CAR. The data support the view that the CA of the brain are essential for CAR. The relative importance of noradrenaline and dopamine is discussed.

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