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Lack of Cross-Tolerance in Rats among (—) Δ^9-*Trans*-Tetrahydrocannabinol (Δ^9-THC), Cannabis Extract, Mescaline and Lysergic Acid Diethylamide (LSD-25)*

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Summary. Using climbing rope and bar-pressing behavior methods, rats were rendered tolerant to Δ^9-THC, cannabis extract, mescaline and LSD-25. Cross-tolerance experiments showed that rats refractory to Δ^9-THC and cannabis extract were still sensitive to mescaline and LSD-25, and vice-versa.

These results suggest that, in spite of the similarity of the clinical symptoms produced in man by the 3 drugs, Δ^9-THC may have its psychotomimetic effects produced by different mechanisms from those of LSD-25 and mescaline.

Key-Words: Cannabis — Lysergic Acid Diethylamide — Mescaline — Hallucinogens — Psychopharmacology.

There is a striking similarity between the psychotomimetic effects produced in man by marihuana, LSD-25 and mescaline. Jaffe has emphasized this resemblance and Hollister has pointed out that the classical description of Baudelaire of hashish intoxication could well describe LSD-25 or mescaline effects. In general, all 3 drugs cause anxiety, changes in mood, difficulty in thinking and concentration, with ideas flowing constant and rapidly; there is also marked distortion of the sensorium especially in the field of visual and auditory perception.

The similarity of the clinical symptoms in man suggests that the 3 drugs may produce their psychogenic effects through some common mechanism. This hypothesis has been strenghtened, at least for LSD-25

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and mescaline, by the demonstration of cross-tolerance between these 2 drugs in man (WOLBACH *et al.*) and in animals (FREEDMAN and AGHAJANIAN).

Tolerance to mescaline and LSD-25 can be rapidly developed in rats (MAHLER and HUMOLLER; FREEDMANN *et al.*, 1958, 1964), and recently CARLINI showed that rats can also be rendered tolerant to marihuana extract it treated long enough.

These facts led us to undertake the present experiments in order to verify a) whether rats could be made tolerant to (-) Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC), and b) whether cross-tolerance could be observed between Δ^9-THC, cannabis extract and LSD-25 and mescaline.

Methods

Δ^9-THC was obtained from hashish by countercurrent distribution according to O'KEEFE (Automatic assembly Quickfit & Quartz, 200 cells, 10 ml upper and lower phase either, solvent system: petroleum-ether / methanol / dimethylformamide / water, 10 : 8 : 2 : 1). After evaporation and extraction of the residue with petroleum-ether the Δ^9-THC is isolated as a mobile colourless oil $[\alpha]_D^{25}$-141° (EtOH; c = 3.16 w/w). It is a mixture of Δ^9-THC with 7.9% w/w dimethylformamide, which is held by hydrogen-bonding of the phenolic group. The content of dimethylformamide was measured by integration of NMR-signals and titration of the THC with lithium isopropylate in dimethylformamide. To remove the amide the mixture is dissolved in the 50—100 fold amount of CCl_4 and shaken twice with water. The organic layer is separated and the solvent removed under reduced pressure in the cold. The resulting viscous oil darkens soon on exposure to air and light. It corresponds in respect of all spectral and physical attributes to the values given in the literature.

Mescaline sulfate (Sigma Chemicals Company) and LSD-25 (Delysid, Sandoz Pharmaceuticals) were used. An alcoholic extract from cannabis grown in the north-east of Brazil (Alagoas state) and a control solution of 0.9% NaCl were prepared and used as described by CARLINI and KRAMER. One mg of dried extract corresponded to 10.3 mg of top flowerings.

Tolerance in rats was studied by 2 methods:

Climbing Rope Studies. The method of WINTER and FLATAKER was used with minor modifications (CARLINI *et al.*). Fifty four male Wistar rats, 3 months old, weighing about 150 g, were trained to climb a vertical rope 160 cm long up to a platform. On reaching the top they were allowed 30 sec food reward. Throughout the experiment the animals were fed for 2 hours daily and were deprived of food 20 to 24 hours before any

experimental session. When all animals were able to negotiate the rope in less than 7 sec, they were submitted to 4 preliminary training sessions in which they were injected with 2.0 ml/kg of control solution and their performances in the rope measured in seconds just before and 30, 60, 90, 120 and 180 min after the injections. This scheme was also followed during the drug-treatment phase.

The rats were then randomly divided in 4 groups for chronic treatments. The first group of 13 animals received 9 daily injections of 40 mg/kg mescaline followed by 2 doses of 10 mg/kg Δ^9-THC. The second group of 12 rats received first 22 injections of 10 mg/kg of Δ^9-THC, followed by 2 doses of 40 mg/kg mescaline; then, after resting 8 days without injections, these animals were injected with one more dose of Δ^9-THC. The third group of 10 rats received 18 injections of cannabis extract, but on days immediately before and after this treatment the animals received one dose of 40 mg/kg of mescaline. The remaining 19 rats were used as controls for the 3 other groups and were therefore injected with the control solution.

All injections were administered daily by the intraperitoneal route, but the trials were run either every day or every second or third days. If an animal failed to reach the food after 1 min because of drug action, this was considered as a failure and the trial was scored as 60 sec.

In all trials the average climbing-time for the control group was calculated. The climbing-times of each drug-treated rat in each experimental session were plotted on graphs: the ordinate represented the climbing-time seconds (1 sec equal to 0.5 cm) and the abscissa the time after injections in minutes (1 hour equal to 5 cm). The areas thus obtained, circumscribed by the curves of the control group and each drug-treated animal, were expressed in cm^2, and the average value plus the standard deviations were calculated. This allowed the expression in cm^2 of the differences in performance between control and drug-treated groups in each experimental session.

Operant Behavior Studies. Four male Wistar rats, 3 months old and deprived of water for 24 hours, were trained in a Skinner-box on a variable interval (VI) schedule to press a bar; 0.02 ml of water was used as reinforcer. The interval used ranged from 10 to 60 sec with an average of 22 sec; training sessions of 30 min each were given daily and sometimes on every second or third days. After the establishement of the baseline performance the animals were injected on the following day with 2.0 mg/kg of control solution five minutes before the experimental session. After this preliminary period, 2 of the animals received daily 130 µg/kg of LSD-25 and the other two 10 mg/kg of Δ^9-THC with no further modification to the method. After development of tolerance to one drug, administration of the other was started.

Results

Cross-Tolerance Studies between LSD-25 and Δ^9-THC. During this work a report from ISBELL *et al.* was published showing that Δ^9-THC was fully active in 10 patients tolerant to LSD-25. Our experiments on these drugs were therefore restricted to the experiment described below.

Operant Behavior Test. Two rats received 130 μg/kg of LSD-25. One of them was partially resistant; from a previous baseline of 340 responses in 30 min (11.3 resp./min), the drug decreased the performance down to 221 responses (7.3 resp./min). The second animal (baseline 371 responses; 12.3 resp./min) was clearly affected showing a complete disruption of performance in the 15 initial minutes and near normal bar pressing behavior during the rest of the experimental session: the rate of response throughout the session was 5.3 resp./min. At the third daily injection of LSD-25 both animals showed tolerance; their rate of bar-pressing was 10.2 and 11.5 resp./min respectively. On the 4th day the rats received 10 mg/kg of Δ^9-THC; there was then a total disruption of response.

Two other rats, with baseline of 9.7 and 12.6 resp./min, were injected daily with 10 mg/kg of Δ^9-THC. During the first 15 injections the animals' performance was strongly depressed by the drug: their rate of response was zero in most cases and was never greater than 3 resp./min. From the 16th injection onwards their performance became increasingly better up to the 28th injection when tolerance was complete in the first rat and partial in the other (9.7 and 8.9 resp./min respectively). On the 29th day they received 200 μg/kg of LSD-25 which reduced the rate of bar-pressing to 0.2 and 0.9 resp./min respectively. Fig. 1 shows the curves obtained with the first animal which exhibited full tolerance.

On the 30th day these animals were still refractory to Δ^9-THC (9.9 and 9.2 resp./min respectively). On the 31st day they were used for a cross-tolerance study with mescaline (see below).

Cross-Tolerance Studies between Mescaline and Δ^9-THC

Operant Behavior Test. A dose of forty mg/kg of mescaline was given to the two rats which had been rendered tolerant to Δ^9-THC, and 30 min later the animals were placed in the Skinner box for 30 min; an almost complete disruption of bar-pressing performance was noted (0.3 and 0.9 resp./min respectively).

Climbing Rope Test. Nine out of the 12 rats treated with 10 mg/kg of Δ^9-THC developed tolerance after 22 daily injections; on the 23rd and 24th days these animals received 40 mg/kg of mescaline. As seen in part A of Fig. 2, the rats tolerant to Δ^9-THC reacted to mescaline, showing no cross-tolerance between both drugs. On the other hand, tolerance to Δ^9-THC was not observed in 3 out of the 12 rats; in these mescaline was

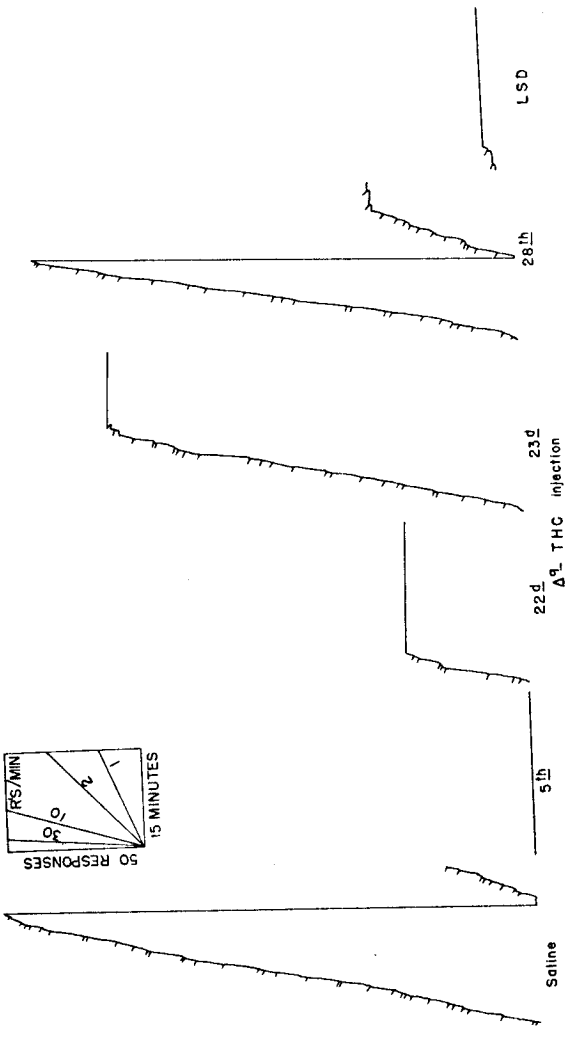

Fig. 1. Typical cumulative response curves for a single rat working on VI 22 for water reward. After 28 daily injections of 10 mg/kg of Δ^9-THC the animal was refractory to the drug but reacted to 200 µg/kg of LSD-25, which shows lack of cross-tolerance between both drugs

also active (Fig. 2, part B). Part A of Fig. 2 shows also that tolerance to Δ^9-THC, once established, persisted for at least 8 days after the drug was discontinued (session n° 11). Fig. 2 shows also the results from the 13 rats treated initially with mescaline followed later by Δ^9-THC. Tolerance to mescaline was complete in 8 rats after 9 daily injections; in them Δ^9-THC produced clear impairment of climbing performance (part C). Five out of the 13 rats, however, did not react to the first 3 daily injections of mescaline; in these mescaline-resistant animals, Δ^9-THC was very active (part D).

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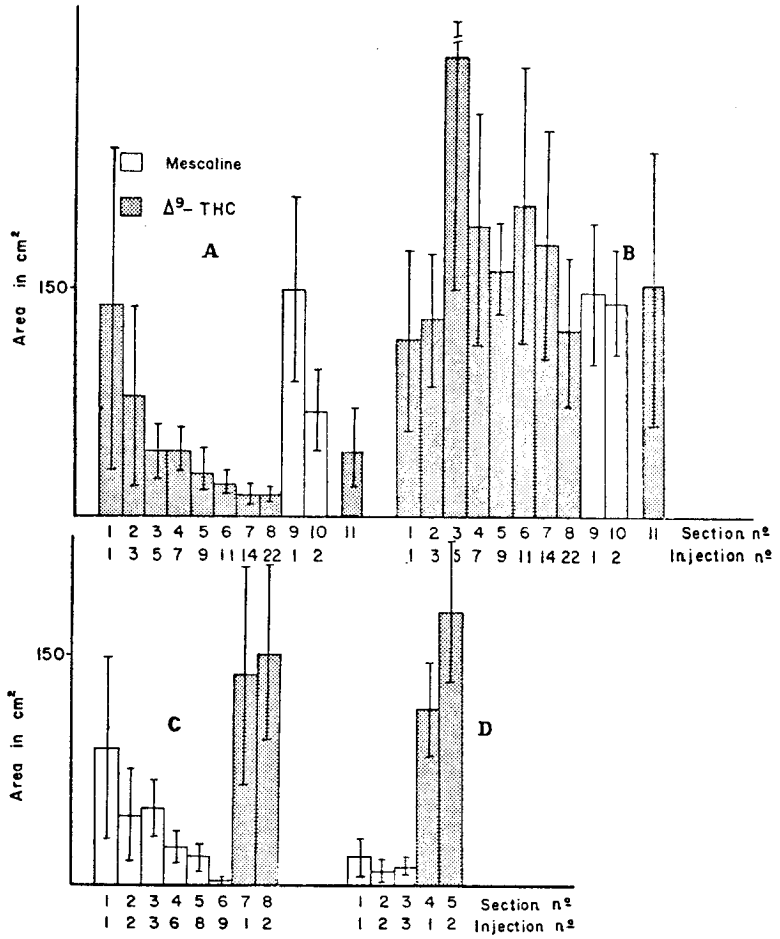

Fig. 2. Lack of cross-tolerance between Δ^9-THC and mescaline, as measured by the climbing rope test. The vertical lines indicate standard deviations. Nine rats tolerant to Δ^9-THC (*A*) and three rats in which tolerance to Δ^9-THC was not developed after 22 daily injections (*B*), responded well to mescaline. In eight rats rendered tolerant to mescaline (*C*) and five animals which were not sensitive to the drug (*D*), Δ^9-THC produced clear impairment of climbing rope performance

Cross-Tolerance Studies between Mescaline and Cannabis Extract

Climbing Rope Test. Fig. 3, which represents the climbing rope performance of 10 rats, shows that mescaline effects before and after development of tolerance to 18 daily injections of extract were practically the same; this again indicates an absence of cross-tolerance between both drugs.

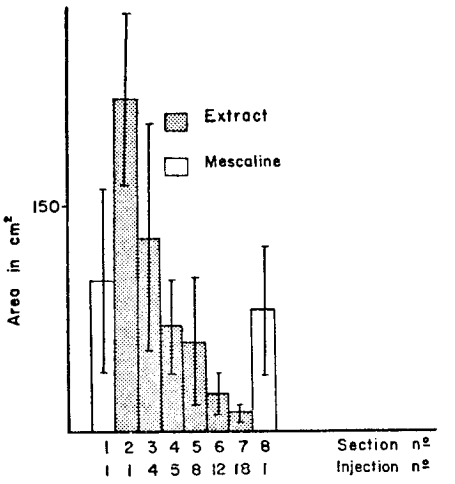

Fig. 3. Lack of cross-tolerance in rats between mescaline and a cannabis extract, as measured by the climbing rope test. The vertical lines indicate standard deviations. (See text for details)

Discussion

The data of the present paper indicate that rats can be rendered tolerant to Δ^9-THC. A similar phenomenon has previously been reported with a marihuana extract (CARLINI). Thus, by the climbing rope test 9 out of 12 rats became refractory to Δ^9-THC after 22 daily injections, whereas by the operant behavior test the 2 rats became tolerant after 29 injections. That tolerance might not be obtained in 100% of the animals has already been shown by CARLINI who obtained tolerance in 7 out of 10 rats injected daily, for a period of 16 days, with 20 mg/kg of a cannabis extract. However, the variability in sensitivity of rats to cannabis must be great, because in the present work all 10 rats injected with the extract became tolerant.

We have observed, on gross inspection, that the symptoms showed by the rats following the administration of Δ^9-THC, mescaline and LSD-25, were very discrete, with neither the climbing rope nor the operant behavior techniques was it possible to distinguish any remarkable effect which was characteristic of any of the 3 drugs. However, abdominal disconfort, inferred from abdominal contractions, after mescaline and Δ^9-THC, and vasodilatation in the ears and anterior paws after mescaline, were noted. As we have described before for mescaline (CARLINI *et al.*) no tolerance to these effects occurred. On the other hand, the sensitivity of our animals to mescaline and LSD-25 was low when compared with the data of FREEDMAN *et al.* (1958, 1964). Thus, in this and in a previous study (CARLINI *et al.*) we have used 40 mg/kg of mescaline and even with

this dose 5 rats were insentitive to the drug. The best effect with LSD-25 was obtained in our rats only with 200 μg/kg, whereas FREEDMAN *et al.* (1958, 1964) used respectively 10 mg/kg and 130 μg/kg. This wide difference in sensitivity of rats to mescaline has been reported before (SMYTHIES *et al.*).

The absence of cross-tolerance between Δ^9-THC on one hand, and mescaline and LSD-25 on the other, deserves further attention. As already mentioned in the introduction, the 3 drugs produced in man similar effects; furthermore, ISBELL *et al.* have very recently reported that their patients stated that Δ^9-THC produced effects similar to those of LSD-25. However, contrary to the observed effect between LSD-25 and mescaline, the present experiments show that Δ^9-THC and cannabis extract induce cross-tolerance with neither of the 2 drugs. These facts may indicate that of the 3 psychotomimetics, Δ^9-THC probably acts by a different mechanism from the other two. The cross-tolerance between LSD-25 and mescaline and the similarity of changes in blood and brain amines produced by both substances are, as stressed by GIARMAN and FREEDMAN, strongly suggestive that they may act through common receptor sites and/or some other similar mechanism which differs from that responsible for Δ^9-THC effects.

Our data on LSD-25 and Δ^9-THC confirm the recent result of ISBELL *et al.* who observed that 10 patients who were tolerant to LSD-25 did not show cross-tolerance to Δ^9-THC.

References

CARLINI, E. A.: Tolerance to chronic administration of Cannabis sativa (marihuana) in rats. Med. Pharmacol. exp. (Bonn) (in press).

—, and C. KRAMER: Effects of Cannabis sativa (marihuana) on maze performance of the rat. Psychopharmacologia (Berl.) **7**, 175—181 (1965).

— M. TERESA A. SILVA, L. C. CESARE, and R. M. ENDO: Effects of chronic administration of β-(3,4-dimethoxyphenyl)-ethylamine and β-(3,4,5-trimethoxyphenyl)-ethylamine on the climbing rope performance of rats. Med. Pharmacol. exp. (Bonn) **17**, 534—542 (1967).

FREEDMAN, D. X., J. B. APPEL, F. R. HARTMAN, and M. E. MOLLIVER: Tolerance to behavioral effects of LSD-25 in rat. J. Pharmacol. exp. Ther. **143**, 309—313 (1964).

—, and G. K. AGHAJANIAN: Time parameters in acute tolerance, cross-tolerance, and antagonism to psychotogens. Fed. Proc. **18**, 390 (1959).

— — E. M. ORNITZ, and B. S. ROSNER: Patterns of tolerance to lysergic acid diethylamide and mescaline in rats. Science **127**, 1173—1174 (1958).

GIARMAN, N. J., and D. X. FREEDMAN: Biochemical aspects of the action of psychotomimetic drugs. Pharmacol. Rev. **17**, 1—25 (1965).

HOLLISTER, L. E.: Chemical psychoses. Ann. Rev. Med. **15**, 203—214 (1964).

ISBELL, H., C. W. GORODETZSKY, D. JASINSKY, U. CLAUSSEN, F. v. SPULAK, and F. KORTE: Effects of (—) Δ^9-trans-tetrahydrocannabinol in man. Psychopharmacologia (Berl.) **11**, 184—188 (1967).

JAFFE, J. H.: Drug addiction and drug abuse. In: The pharmacological basis of therapeutics, p. 285. Ed. L. S. GOODMAN and A. GILMAN. Third edition. New York: MacMillan 1966.

MAHLER, D. J., and F. L. HUMOLLER: Effect of lysergic acid diethylamide and bufotenine on performance of trained rats. Proc. Soc. exp. Biol. (N. Y.) **102**, 697—701 (1959).

SMYTHIES, J. R., E. A. SYKES, and C. P. LORD: Structure-activity relationship studies on mescaline. II. Tolerance and cross-tolerance between mescaline and its analogs in the rat. Psychopharmacologia (Berl.) **9**, 434—446 (1966).

WINTER, C. A., and L. FLATAKER: Effects of lysergic acid diethylamide upon performance of trained rats. Proc. Soc. exp. Biol. (N. Y.) **92**, 285—289 (1956).

WOLBACH, A. B. JR., H. ISBELL, and E. J. MINER: Cross-tolerance between mescaline and LSD-25 with a comparison of the mescaline and LSD reactions. Psychopharmacologia (Berl.) **3**, 1—14 (1962).

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