Psychopharmacologia (Berl.) 9, 434—446 (1966)

Structure-Activity Relationship Studies on Mescaline:

II. Tolerance and Cross-Tolerance between Mescaline and its Analogues in the Rat

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Received November 29, 1965

In previous communications (Smythies and Sykes, 1964, 1965), the effect of mescaline hydrochloride on the conditioned avoidance response in the rat has been analysed, together with the comparative effect of some of its structural analogues. Using a modified Levine Shuttle box with sound as the conditioned stimulus and shock as the unconditioned stimulus, it was found that mescaline had a biphasic effect, larger doses causing an initial increase in CAR inhibition followed by excitation; in the main, smaller doses produce only excitation. Different animals showed widely differing sensitivities to mescaline, and two groups of rats were delineated, Class I and Class II. At a dose of 25 mg/kg mescaline i.p., Class I showed an overall inhibitory effect and Class II showed an overall excitatory effect. However, at a dose of 12.5 mg/kg, Class I rats behave like Class II rats at 25 mg/kg and Class II rats show little reaction. This suggests that the difference is purely quantitative and merely expresses a difference in sensitivity among the animals.

The effect of two analogues of mescaline, 3.4-dimethoxyphenylethylamine and N:N-dimethyl mescaline, were also studied using the conditioned avoidance reaction (Smythies and Sykes, 1965). The former had predominantly an inhibitory effect (manifested as a typical "double-hump" curve), and the latter an excitatory effect at the doses used (25—100 mg/kg i.p.).

It was decided to carry out cross-tolerance studies on these drugs in the hope that any interaction between them would provide further information on the precise mode of action of each drug.

Method

Subjects. 32 experimentally naive male hooded rats, supplied by the National Institute of Medical Research and by Animal Suppliers, London, were used in this study. The animals were approximately 16—20 weeks old at the beginning of the experiment, and were allowed food and water ad libitum. For each cross-tolerance experiment, naive rats were used.

Apparatus. A modified Levine Shuttle box was used, which has been described in detail in an earlier paper (Smythies and Sykes, 1964). An

animal was able to terminate the conditioned stimulus (CS) or the unconditioned stimulus (UCS) by crossing from one compartment to the other.

The inter-trial intervals and the duration of the CS and UCS were controlled automatically by a programming unit placed in an adjacent room.

Procedure. In the investigation, the same technique was used as in the earlier studies (SMYTHIES and SYKES, 1964). The basic response required of an animal in the test situation was a cross from one side of the box to the other in response to a given stimulus. The conditioned stimulus (a buzzer) sounded for 5 sec, at the end of which time the unconditioned stimulus (electric shock of 1.0 m.a.) was presented if the animal had failed to respond to the CS. Each experimental period of 2 hrs consisted of seven runs of 20 trials each, the runs being separated by a time interval of 5 min. The results were measured as follows: the number of shocks received (escape responses) and hence the number of shocks avoided (avoidance responses), the number of crosses made (an activity measure) and the reaction time, which is the interval between the onset of the CS and its termination by the animal measured in tenths of a second on a print-out counter. After training to a criterion of 80—85% avoidance, intraperitoneal injections of either drug or saline were given after 40 trials, i.e. after the first two runs of 20 trials each. The experiment then continued as before. The results of the first 40 trials were discarded, since they were included originally to allow the animal to become acclimatised to the experimental situation.

In the cross-tolerance studies, the following experimental design was adopted:

Day 1: Drug A: first dose (preceded the day before and followed the two successive days by saline controls).

Day 14: Pre-drug saline. Days 15—21 (inclusive): Drug B, one dose daily. Day 22: Drug A: second dose. Days 23, 24: Post-drug salines. For Drug A, the first and the second dose were identical; for Drug B, the same dose was given each day.

This experimental design had the advantage of providing information both on the development of tolerance to Drug B itself and on the development of cross-tolerance (if any) between Drugs A and B. Previous studies have shown that little or no tolerance effect develops if doses of mescaline are separated by 2 weeks. Hence, any tolerance exhibited during the second administration of Drug A must be due to cross-tolerance to Drug B.

The levels of drugs given were based on results from previous studies (SMYTHIES and SYKES, 1964, 1965). As before, the results for each run of 20 trials are expressed in D-S scores (derived as described in SMYTHIES and SYKES, 1965), i.e. the difference between the drug score and the mean of the pre-drug and post-drug saline scores.

Analysis of results. Non-parametric statistics were used owing to the non-normal distribution of the results, due, in part, to individual variation and to variation in any one animal's behaviour from day to day. Wilcoxon's method for paired replicates was employed.

Results

1. Mescaline tolerance 25 mg/kg

The developmental pattern of tolerance to mescaline itself was examined first, the data being obtained from the cross-tolerance studies. Figs. 3 and 6 show the mean reaction times in D-S scores, each block in

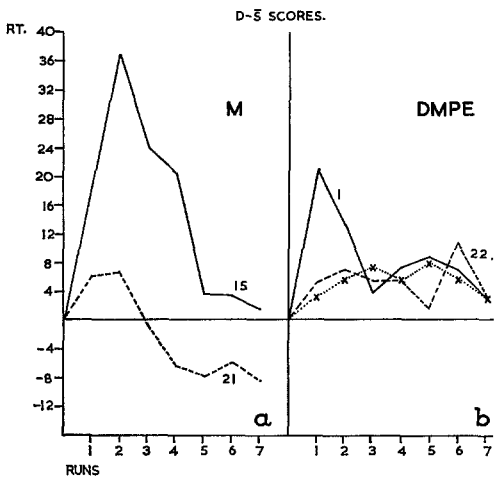

Fig. 1 a and b. Cross-tolerance between mescaline and DMPE. *Abscissa:* Blocks of runs of 20 trials each. The interval between runs represents 13 min in each case (run 8 min, 5 min time out). *Ordinate:* Mean change in reaction time in seconds (D—$\bar{S}$) each point represents 160 readings (20 trials $\times 8$ animals per run) averaged. a 1st (day 15) and 7th (day 21) mescaline run compared; b Pre- and post-mescaline tolerance 3.4-dimethoxyphenyl ethylamine (DMPE) runs compared (i) —·—·— pre-tolerance at 50 mg/kg DMPE; (ii) - - · · · - - post-tolerance at 50 mg/kg DMPE; (iii) ··· × ··· × ··· pre-tolerance at 25 mg/kg DMPE. p (runs 1—3) < 0.02. p (runs 1—7) n.s. for difference between (I) and (II). Class I rats, 6: Class II rats, 2

the histogram representing the total change in reaction time for all 7 runs for each day. Fig. 3 illustrates the rapid development of tolerance to the inhibitory effect of mescaline, with the development of an accentuated excitatory phase. In Fig. 1 a the time course of the mescaline effect on the first day is compared with that on the final or seventh day. This degree and type of change resembles, to some extent, that induced by halving the dose of mescaline from 25—12.5 mg/kg with a fortnight's interval.

The mescaline effect in Fig. 6 is similar to that in Fig. 3, although the initial inhibitory effect is less, and the subsequent excitatory score greater. This difference between the mescaline effects in Figs. 3 and 6 may be accounted for by the fact that the first population in Fig. 3 contained

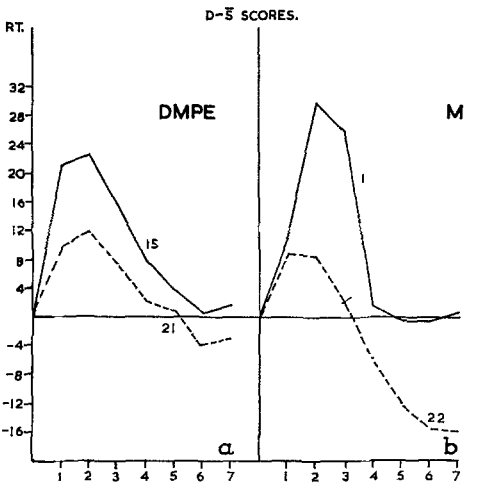

Fig. 2 a and b. Cross-tolerance between DMPE and mescaline. *Abscissa:* Blocks of runs of 20 trials each. The interval between runs represents 13 min in each case (run 8 min, 5 min time out). *Ordinate:* Mean change in reaction time in seconds (D—$\bar{S}$) each point represents 160 readings (20 trials × 8 animals per run) averaged. a 1st (day 15) and 7th (day 21) mescaline run compared; b As 2a for mescaline. (Class I rats, 5; Class II rats, 3)

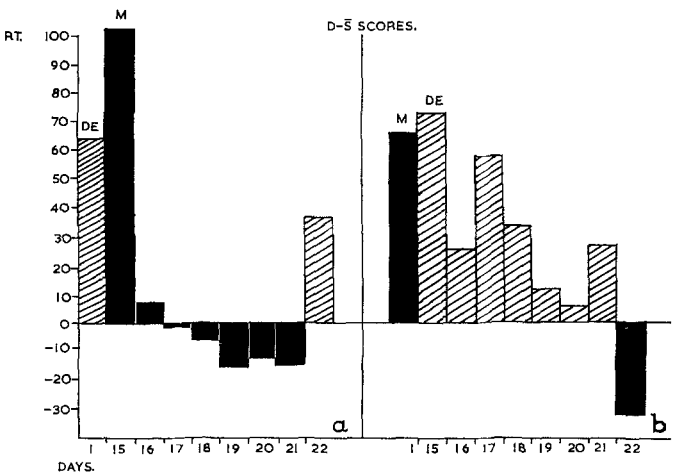

Fig. 3 a and b. Histograms to represent progress of tolerance and cross-tolerance for mescaline and DMPE. *Abscissa:* Days of experiment. *Ordinate:* Total mean D-$\bar{S}$ change in seconds for all 7 runs for the day for each day of drug administration. a Solid bars in each case: Mescaline (M); b Hatched bars: 3,4-dimethoxyphenylethylamine (DMPE)

six Class I animals (most sensitive to mescaline) and two Class II (least sensitive), while the population in Fig. 6 contained 4 of each class. Nevertheless, Fig. 6 also demonstrated the lack of tolerance to the excitatory phase, which shows a steady increase. Again, the effect of reducing the original mescaline dose by half in a Class II population is simulated.

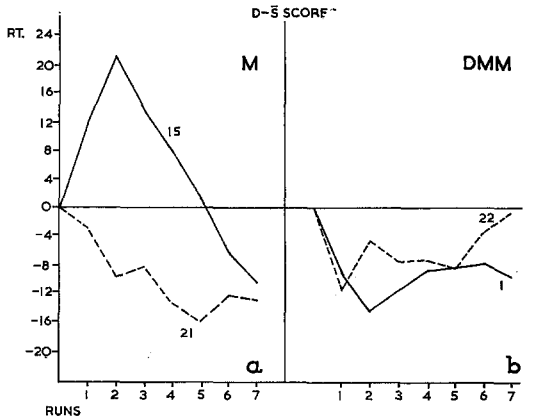

Fig. 4. a Cross-tolerance between mescaline and N,N-dimethylmescaline (DMM). Arrangements as for Fig. 4. b $p < 0.02$ for all 7 runs. Class I rats, 4: Class II rats, 4

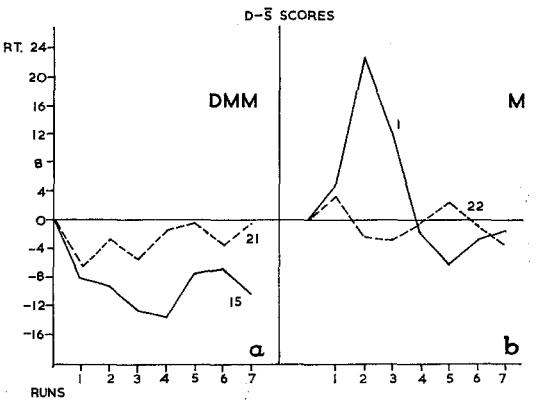

Fig. 5a and b. Cross-tolerance between DMM and mescaline. Arrangements as for Fig. 1. a $p < 0.01$; b p (runs 5—6) n.s. Class I rats, 3: Class II rats, 6

In order to investigate Classes I and II separately, the mescaline scores from all animals composing the populations in Figs. 3 and 6 were re-grouped; the Class I group ($n = 10$) were then compared with the Class II group ($n = 6$). The results of this re-grouping are given in Fig. 7. In Class I, there is a very rapid development of tolerance to the inhibitory effect of mescaline, but a complete lack of tolerance development is shown to the excitatory action of the drug in either class; there is, if anything, a slight increase in this excitatory effect on successive days.

2. Cross-tolerance between 3.4-dimethoxyphenylethylamine (DMPE) (drug A; 50 mg/kg) and mescaline (drug B; 25 mg/kg)

In the first cross-tolerance experiment, drug A was 3,4-dimethoxyphenylethylamine (DMPE) in doses of 50 mg/kg, and drug B was mesca-

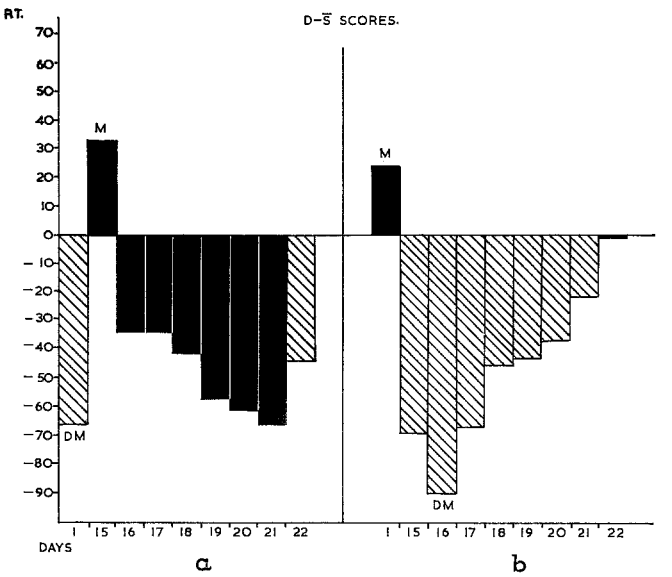

Fig. 6a and b. Arrangements as for Fig. 3, for mescaline and N,N-dimethylmescaline (DMM)

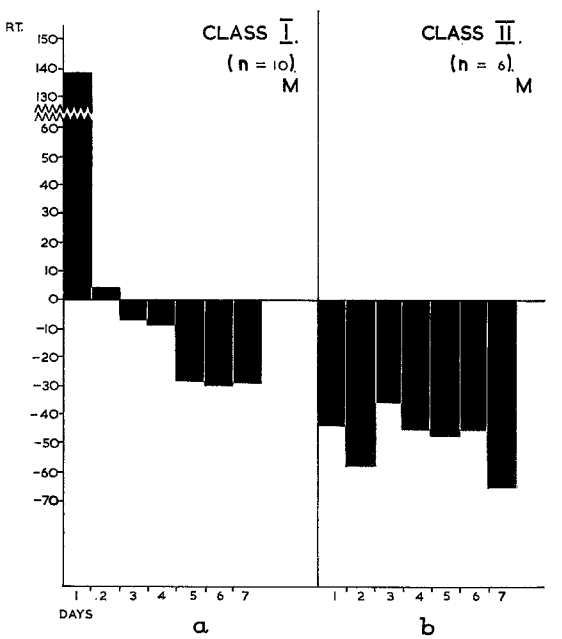

Fig. 7a and b. As for Fig. 3. a shows development of tolerance to mescaline in Class I rats ($n = 10$) and b in Class II rats ($n = 6$) to mescaline

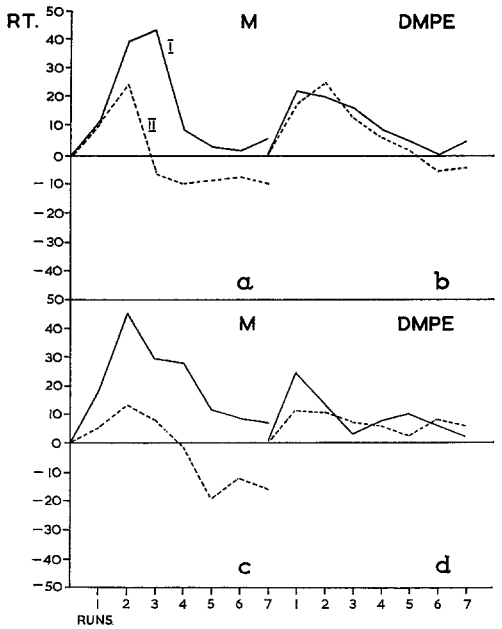

Fig. 8a—d. As Fig. 1, comparing Class I and Class II reactions to mescaline (a) and the same rats reaction to DMPE (b) and likewise for c and d for another population

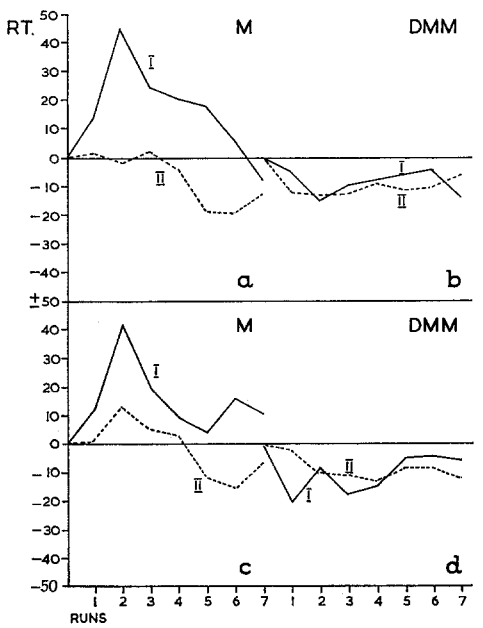

Fig. 9a—d. As Fig. 1, comparing Class I and Class II reactions to mescaline (a) and the same rats reaction to DMM (b) and likewise for c and d for another population

line at 26 mg/kg. The results are shown in Figs. 1 and 3a. It can be seen that the pre-mescaline effect of DMPE shows an overall inhibitory effect with a bimodal peak distribution. Following the administration of mescaline for 7 days, the post-mescaline DMPE curve shows that a degree of cross-tolerance exists between the two drugs (Fig. 3a). When the first three points of the pre-and post-mescaline DMPE curves were compared, it was found that the first peak was reduced ($p > 0.02$), whereas the second peak remained prominent (p.n.s.) (Fig. 1b). For the sake of comparison this figure also shows the effect of 25 mg/kg DMPE given to the same population of rats at an earlier date; it is apparent that there is a marked similarity between this curve and the post-mescaline DMPE effect at 50 mg/kg.

3. Cross-tolerance between mescaline (drug A; 25 mg/kg) and 3.4-dimethoxyphenylethylamine (drug B; 50 mg/kg)

The administration of the two drugs was reversed in the second cross-tolerance experiment, although the same dose levels as before were used. The results (see Figs. 2 and 3b) show that the development of tolerance to the initial inhibitory effect of DMPE is considerably slower than that for mescaline. The significance of a second peak which appeared at days 17 and 18 requires further investigation, but it is possible that it is connected with the bimodal distribution of the inhibitory effect of DMPE, which has been reported elsewhere (SMYTHIES and SYKES, 1965). However, only two animals showed this bimodal effect (Fig. 1b), which suggests that individual differences between rats play a role here as elsewhere. Fig. 2a shows the time course of the development of tolerance to DMPE, from the first DMPE day (day 14) to the 7th DMPE day (day 21); a small "excitatory" phase has appeared, which may mean that DMPE has a slight excitatory potential if the dominant inhibitory effect is reduced by tolerance. However, there is clearly marked cross-tolerance between DMPE and mescaline.

4. Cross-tolerance between N.N-dimethylmescaline (DMM) (drug A; 50 mg/kg) and mescaline (drug B; 25 mg/kg)

In this case, Drug A was N,N-dimethylmescaline (50 mg/kg) and Drug B was mescaline (25 mg/kg). The results of this experiment are shown in Figs. 4 and 6a. The expected excitatory effect of DMM was shown, and cross-tolerance was demonstrated between the two drugs. Tolerance to the inhibitory effect of mescaline was again rapid (Fig. 6a), and the excitatory effect of this drug was increased. However, despite this lack of tolerance produced by mescaline to its own excitatory action, it is interesting to note that it nevertheless induces cross-tolerance for the excitatory effect of DMM.

5. Cross-tolerance between mescaline (drug A; 25 mg/kg) and dimethylmescaline (drug B; 50 mg/kg)

The fourth experiment was a comparison between mescaline as Drug A (25 mg/kg) and DMM as Drug B (50 mg/kg), the results of which are shown in Figs. 5 and 6b. The response to repeated doses of DMM shows that an increase in its excitatory effect on the second day is followed by a steady decrease for the subsequent 5 days (Fig. 6b). Fig. 5a compares the time course for the first DMM day with the seventh, the decrease in excitatory effect being highly significant ($p > 0.01$).

There is also cross-tolerance between DMM and mescaline (Fig. 5b); the inhibitory effect of mescaline is reduced, and the later excitatory effect (which tends to be prominent at this stage) is almost abolished (cf. Fig. 6a). The differences in the pre- and post-DMM scores for runs 5 and 6 do not reach statistical significance, and there is an obvious difference between the post-drug curves for mescaline after DMPE (Fig. 2b) and after DMM (Fig. 5b); statistical comparison in this instance would not be particularly meaningful as two different populations of rats are involved.

These results suggest that mescaline does not induce tolerance for its own excitatory effect (in the time course studied), whereas DMM clearly does induce tolerance for its own excitatory effect; moreover, this latter tolerance may exert cross-tolerance for the excitatory effect of mescaline. It is worth noting that the mescaline curves for the pattern of development of its own tolerance is compounded of a mixed population of Class I and Class II rats. The data in Fig. 7, however, shows that neither class develops tolerance to the excitatory effect.

6. Individual sensitivity to the three drugs

It was felt that it would be worth investigating the possibility that those rats sensitive to mescaline (Class I) might also be sensitive to DMPE and DMM. Figs. 8 and 9 clearly indicate that such differential sensitivity did not exist. The degree of sensitivity to mescaline remained a relatively permanent feature for each animal, insofar as those that were originally sensitive remained so on a future occasion, providing that at least 2 weeks separated the two drug administrations to obviate any tolerance effects.

Discussion

The results of these experiments show that cross-tolerance exists between mescaline and both DMPE and DMM in both directions. However, the nature of these results gives rise to two major problems, namely quantification and hence, to some extent, interpretation.

Problem of quantification

It might appear at first that the cross-tolerance between mescaline and DMPE (Figs. 1 and 3a) is less than that between DMPE and mesca-

line (Figs. 2 and 3b). Thus, it seems that rats made tolerant to mescaline show some cross-tolerance to DMPE (reduction of the mean D-$\bar{S}$ score from + 64 to + 37) while rats made tolerant to DMPE are very tolerant to mescaline (reduction of the mean D-$\bar{S}$ score from + 66 to − 32). The degree of cross-tolerance could possibly be measured by determining the total number of days of tolerance development required to reach the observed cross-tolerance level.

The large swing in the pre- and post-drug mescaline scores in Fig. 3b may be related to the fact that tolerance to mescaline develops very quickly. It is possible to say that this degree of swing would be equivalent to the change one might expect if the 7 days of DMPE had been replaced by 2, 3 or 4 days of mescaline. Again, the change in the DMPE score shown in Fig. 3a is apparently small, but this must be evaluated against the fact that, as Fig. 3b shows, tolerance to DMPE develops slowly and somewhat erratically.

Similarly, the change in the pre- and post-DMM scores for mescaline (Fig. 6b) is complex. Self-tolerance to the excitatory and inhibitory effect of mescaline seems to develop independently when the results of experiments with Class I and Class II animals are considered. Tolerance to both effects appears if mescaline is given to a DMM-tolerant animal, although tolerance to the excitatory effect is not noticable in mescaline self-tolerance. The change in the pre- and post-mescaline scores for DMM (Fig. 6a) shows a decrease roughly equivalent to that produced by 4—6 days of DMM itself (Fig. 6b), although this result was obtained from another sample.

Therefore, from these preliminary studies, it seems clear that in order to quantify cross-tolerance studies as effectively as possible, it would be advisable: to use a uniform population of rats in terms of sensitivity to mescaline, i.e. all Class I and/or all Class II (possibly with sub-divisions within the two classes) and to vary the number of days on which drug B is given. Thus, for example, the rapid development of tolerance to the inhibitory effect of mescaline in Class I rats suggests the following design:

a) Day 1: 25 mg/kg mescaline to determine class;

b) Day 22: select all Class I animals and administer drug B *once*;

c) Day 23: 25 mg/kg mescaline.

Other designs could be developed to measure, for example, cross-tolerance to the excitatory effect, using a Class II population.

Problem of interpretation

The excitatory effect of mescaline must be due to central CNS factors, but it cannot be assumed that the "inhibitory" effect is necessarily so.

Behavioural inhibition of a reaction to a central stimulus may be produced by direct interference with the mechanisms of learning and conditioning. On the other hand, some other central factors such as nausea, some peripheral factor (for example muscle weakness), or a combined effect from the unpleasant viscero-sensory results from excessive peripheral autonomic activity, may be operative.

There is no doubt that mescaline in a dose of 50 mg/kg induces obvious muscle weakness, and it has been shown by SCHOPP *et al.* (1961) to have a direct curare-like effect on muscle. However, at 25 mg/kg the animals showed no objective signs of paralysis and their motor reactions to unconditioned stimuli appeared relatively normal. An attempt was made to obtain a quantitative assessment of any ataxic effects by using RUSHTON and STEINBERG's (1963) ataxia test. This showed that at 25 mg/kg no ataxia was produced, as the amount of splay between the foot-prints remained constant; however, the length of the steps was significantly shorter under the influence of mescaline than under saline ($p > 0.05$).

It is interesting that mescaline is capable of producing intense nausea in humans. Therefore, possibly the rat does not react to the conditioned stimuli merely because it is feeling ill. It will be recalled that many behavioural responses, including learned responses, may be affected by stimulation of limbic structures. In some of these cases, it appears probable that the learning or conditioning mechanism itself has not been affected, but that emotional reactions (such as pleasure or fright) have been induced by stimulation of Olds' positive or negative areas. Thus, the animal may be unresponsive to the CS merely because its attention is distracted by sensations of pleasure or fear which have been centrally induced. In the case of mescaline, the rats gave no objective signs of feeling ill, or of fear, in the sense that the freezing response was not observed; in a few cases, excess salivation occurred, suggesting that there may have been some peripheral autonomic stimulation. An animal is seen react to the CS by behavioural signs of attention, but it does not move. In some instances, it may be observed to make a common reaction to mescaline, namely, intense sniffing between the bars of the floor grid almost as though the rat were hallucinated. Since there is no way of measuring "feeling ill," an attempt was made to test the hypothesis that the CAR is inhibited by peripheral factors that are not directly connected with the CAR mechanism. We therefore tested the action on the CAR of two analogues of mescaline that would be expected to have at least as powerful a peripheral action as DMPE (*if* this indeed has any) and yet are unlikely to cross the blood-brain barrier: 3-hydroxy-4-methoxyphenylethylamine and 3-methoxy-4-hydroxyphenylethylamine. When administered at doses of 50 and 100 mg/kg i.p. there was no significant inhibition of the CAR. (The initial apparent deflection with the former compound

at 100 mg/kg does not quite reach statistical significance.) They both at this dose induced an observable increase in respiration rate in some animals (2/2 with the former, 2/4 with the latter). There was no other signs of autonomic activity (salivation, piloerection, or excess sweating). However, this facet of the analysis would require to be carried out under classical pharmacological conditions. We concluded that the inhibition of the CAR which occurs under mescaline is probably not due to its peripheral effects.

Summary

Using the CAR shuttle box technique, with male hooded rats as subjects, the development of tolerance to mescaline, 3,4-dimethoxyphenylethylamine (DMPE) and N:N-dimethylmescaline (DMM) and possible cross-tolerance between these drugs was studied. It was found that seven successive daily doses of mescaline induced tolerance to its "inhibitory" effect, but that the "excitatory" effect was increased. Tolerance developed to the predominantly "inhibitory" effect of DMPE, and to the "excitatory" effect of DMM.

Partial cross-tolerance existed between DMPE and mescaline, and when the procedure was reversed, between mescaline and DMPE. Cross-tolerance was shown in the same manner between DMM and mescaline, and mescaline and DMM.

The problems of quantification and interpretation of these results is discussed. It was concluded, on the basis of subsidiary experiments, that the "inhibitory" effect of mescaline was probably not caused by ataxia or by a peripheral effect.

Acknowledgments. We are grateful to Professor G. M. CARSTAIRS for his continued support of this project and to Dr. J. MARKS of Roche Products Limited for supplies of 3:4-dimethoxyphenylethylamine and N:N-dimethylmescaline. We should also like to thank NORMAN CLARKE and JIM THOMPSON for their technical assistance.

This work was supported by grant number G 964/222/B from the Medical Research Council.

Erratum

In Psychopharmacologia (*8*, 5), in our previous article on the subject please read the formulae on p. 326:
Then

$$D-\bar{S} = \Sigma_{20}^{0}\, x - \sum_{N}^{0} \frac{y+z}{2}$$

Therefore, each point on the graphs $= \dfrac{\Sigma_{20}^{0}\,(D-\bar{S})}{N}$

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