Psychopharmacologia (Berl.) 43, 201–205 (1975) – © by Springer-Verlag 1975

Electroencephalographic Studies on the Development of Tolerance and Cross Tolerance to Mescaline in the Rat*

BRENDA COLASANTI[1] and NAIM KHAZAN[2]

Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York
New York, New York

Received October 24, 1974; Final Version April 17, 1975

Abstract. Recordings of the electroencephalogram (EEG) and the electromyogram (EMG) were collected continuously from rats equipped with permanent cortical and temporalis muscle electrodes. Automatic injections of mescaline were administered through indwelling i.p. cannulas at an initial dose of 30 mg/kg every 6 hrs for the first 2 days. This dose was then increased to 60 mg/kg/6 hr which was given for the duration of the study. The initial injections of the mescaline induced an immediate desynchronization of the EEG and behavioral arousal of the rat, which endured for 2–3 hrs. After this time, slow wave (SW) sleep and rapid eye movement (REM) sleep episodes reappeared, with the return of regular alternations of the sleep-wakefulness cycle. Upon continued administration of the drug, partial tolerance to the arousal effects of mescaline developed, which was reflected by a gradual reduction in the latencies to onset of SW sleep and REM sleep. Rats rendered tolerant to mescaline in this manner were found to be cross tolerant to lysergic acid diethylamide (LSD) and N,N-diethyltryptamine (DET). In contrast, cross tolerance did not occur to amphetamine, which exerts similar arousal and EEG desynchronizing effects. These results agree with physiological and behavioral studies of tolerance and cross tolerance among hallucinogens and support the usefulness of the EEG as a quantitative indicator of central nervous system functions.

Key words: Mescaline – LSD – Amphetamine – EEG – Cross Tolerance – REM Sleep.

The similarity of the constellation of physiological and psychological effects produced in man by the acute administration of hallucinogens containing an indolealkylamine or a phenylethylamine moiety has been well-documented (Hoch *et al.*, 1952; Balestrieri and Fontanari, 1959; Wolbach *et al.*, 1962). Following the chronic administration of these hallucinogens, direct tolerance and cross tolerance develop to many of the sympathomimetic effects and to the behavioral changes as well (Balestrieri and Fontanari, 1959; Wolbach *et al.*, 1962). Laboratory studies in animals have demonstrated that hallucinogens such as lysergic acid diethylamide (LSD) and mescaline given acutely disrupt the performance of previously learned behaviors (Freedman *et al.*, 1958; Smythies and Sykes, 1964). After repeated injections of these drugs, however, tolerance and cross tolerance to these behavioral effects were found to occur (Freedman *et al.*, 1958; Freedman *et al.*, 1964; Appel and Freedman, 1968).

Electroencephalographic (EEG) studies on the acute effects of hallucinogenic drugs in experimental animals have revealed characteristic desynchronization of the EEG tracings with a flattening of amplitude and a decrease of slow activity (Bradley, 1953; Pierre, 1957; Khazan and McCash, 1965). Quantitative integration of the EEG in man after the administration of LSD has likewise demonstrated a reduction in amplitude, as reflected by a 25% decrease in the "mean EEG energy content", or EEG voltage output (Goldstein *et al.*, 1963a). A decrease in the EEG voltage output and its variability has also been observed after the acute administration of amphetamine to experimental animals (Munoz and Goldstein, 1961) and to man (Goldstein *et al.*, 1963b). These changes in the amplitude of the EEG correlated with the central nervous system (CNS) stimulant properties of the hallucinogens and of amphetamine (Goldstein *et al.*, 1963b).

In a limited number of studies, the EEG was recorded during the chronic administration of a hallucinogen. After repeated injections of LSD, tolerance was found to develop to the EEG changes induced in cats (Adey *et al.*, 1962; Gillin *et al.*, 1973). After the repeated administration of N,N-dimethyltryptamine (DMT) to cats, however, tolerance development did not occur (Gillin *et al.*, 1973).

The present study examined the effect of chronic administration of mescaline on the EEG and sleep-

* Supported by NIMH Grants DA 00461 and DA 01050.

[1] Present address: Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia 26506, U.S.A.

[2] Present address: Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, Maryland 21201, U.S.A.

wakefulness cycle of the rat. The latter cycle is comprised of three behavioral states; namely, a synchronized sleep state which is also called slow wave (SW) sleep, an activated sleep state often called rapid eye movement (REM) sleep, and the state of wakefulness. The development of tolerance to the stimulant properties of mescaline was determined by evaluation of the latencies to SW sleep and REM sleep onset produced by successive injections of the drug. Possible cross tolerance was then measured between the mescaline EEG effects and the similar effects of LSD, N,N-diethyltryptamine (DET), and amphetamine.

Methods

Adult female Sprague-Dawley rats weighing 250–300 g at the time of surgery were used in these experiments. Permanent electrodes for polygraphic recording were implanted into a total of 10 rats under pentobarbital (30 mg/kg i.p.) and ether anesthesia. For bipolar recording of the cortical EEG (ECoG), stainless steel screws as electrodes were placed over the frontal and parietal cortices. For bipolar recording of the electromyogram (EMG), stainless steel wires were inserted into the temporalis muscles. Polyethylene cannulas were also implanted i.p. at this time (Weeks, 1972) into the 5 rats to be treated chronically with mescaline. After a period of 1 week for recovery from surgical procedures, the rats were placed in individual EEG recording cages and connected to a cable. Lighting conditions in the laboratory provided a timer-regulated dark period from 10:00 in the evening to 6:00 in the morning. EEG and EMG tracings from these relatively unrestrained and freely moving rats were monitored on a Grass model 7 polygraph and a Beckman type R Dynograph.

During a control period of 2 days, EEG and EMG recordings were collected continuously from the 5 experimental rats during i.p. injections of isotonic saline (0.25 ml once every 6 hrs), delivered automatically by motor driven syringes. Automatic injections of mescaline to the experimental group were then begun at noon (12:00 p.m.). An initial dose of 30 mg/kg was delivered every 6 hrs for the first 48 hrs. This dose was then increased to 60 mg/kg/6 hr, which was given for the duration of the study (15 days).

The development of tolerance to mescaline was assessed by determination of the latencies to onset of SW sleep and REM sleep, respectively, after successive injections of the drug. The criterion used for establishing the onset of both SW sleep and REM sleep from EEG and EMG recordings was the appearance of the first episode of no less than 1 min in duration. REM sleep episodes were identified by the appearance of characteristic low voltage fast EEG activity resembling wakefulness but occurring in association with an extreme reduction in the EMG activity surpassing that of SW sleep. Statistical comparisons of the data obtained on the last day of saline treatment and on each successive drug day were made by Student's 2 tailed *t*-test.

Experiments on cross tolerance to mescaline were begun on the sixth day of chronic mescaline administration. Cross tolerance was determined by the substitution of another drug for the mescaline injection ordinarily given at noon. The test compound was administered simultaneously to 5 control rats. SW sleep and REM sleep onsets after the injection were then determined as above for both groups of rats, and the results were evaluated statistically by Student's *t*-test. The drugs studied in this manner and the doses used were: LSD–100, 200, and 400 µg/kg; DET–5 and 10 mg/kg; and amphetamine–0.625, 1.25, and 2.5 mg/kg. These compounds were given in random order, with at least a 48-hr interval between administrations of the same drug at different doses.

Results

EEG and EMG recordings during the control period of the rats to be treated with mescaline revealed the presence of regular alternations among the three behavioral states of SW sleep, REM sleep, and wakefulness. The latencies to SW sleep onset and to REM sleep onset after the administration of saline did not appear to be related to the saline injection. Instead, extremely short latencies were found to occur after injections given during the daytime hours, while longer latencies were manifest after injections given during the night (Fig. 1).

The administration of mescaline at an initial dose of 30 mg/kg/6 hr was followed, as expected, by EEG activation or desynchrony and by behavioral arousal of the rat. This stimulant effect of mescaline was reflected in the delay to onset of both SW sleep and REM sleep after injection (Fig. 1). By the third day of chronic mescaline administration, when the dose was changed to 60 mg/kg/6 hr, SW sleep and REM sleep onsets after injections were still markedly prolonged. These latencies to onset subsequently became somewhat reduced by the third day after maintenance of the rats at the 60 mg/kg/6 hr dose, but the development of tolerance to the stimulant effect of mescaline at this time was still not complete.

The administration of a 30 or a 60 mg/kg dose of mescaline to the control group of rats induced a delay in the onset of both SW sleep and REM sleep. Rats treated chronically with mescaline and injected simultaneously demonstrated much shorter latencies to SW sleep onset and REM sleep onset (Fig. 2). Administration of LSD at the 100 or the 200 µg/kg dose level likewise resulted in a marked shortening of these latencies in the group of rats chronically treated with mescaline in comparison with the control animals (Fig. 3).

In contrast, the latencies to SW sleep onset and REM sleep onset after the administration of amphetamine were found to be the same in both the control and mescaline-treated groups of rats at the 0.625, 1.25, and 2.5 mg/kg dose levels (Fig. 4). DET administered at the dose of 5 mg/kg likewise produced similar SW sleep and REM sleep onsets in the 2 groups of rats (Fig. 5). At the dose of 10 mg/kg, however, a marked shortening in these times to onset was seen in the mescaline-treated animals.

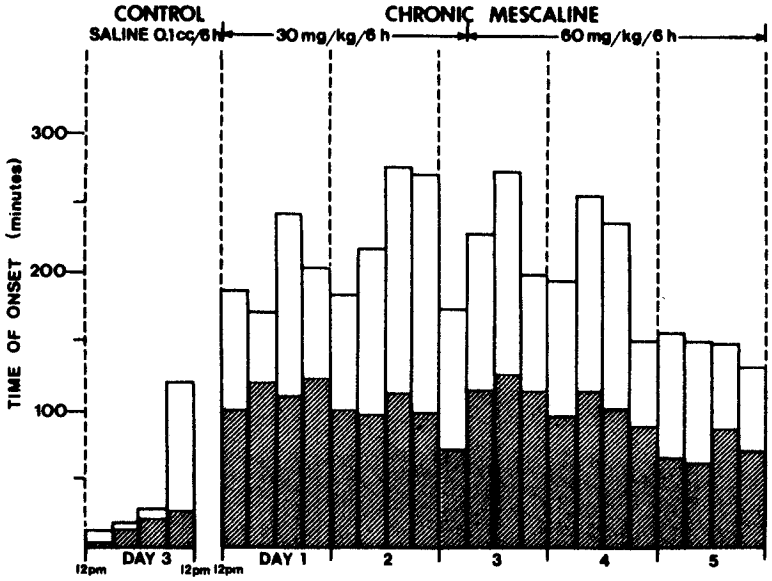

Fig. 1. Latencies to onset of SW sleep (vertical striped bars) and REM sleep (open bars) in the rat after successive i.p. injections of mescaline initiated at noon (12:00 p.m.) and given every 6 hrs. Although the stimulant effect of this hallucinogen gradually decreased, SW sleep and REM sleep onsets after mescaline injections given on the fifth day of the chronic treatment remained significantly elevated over the comparable values obtained after saline administration ($P < 0.01$; $n = 5$ rats)

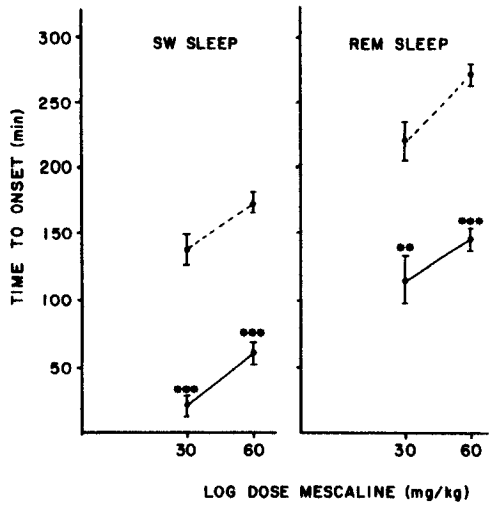

Fig. 2. Latencies to onset of SW sleep and REM sleep after the administration of mescaline at the specified dose levels to control rats (broken lines) and rats treated chronically with mescaline (solid lines). The onset of both SW sleep and REM sleep after each dose occurred much earlier in the rats having prior exposure to mescaline. (Each point represents the mean for 5 rats, while the vertical bars indicate ± S.E.M.; ** $P < 0.01$; *** $P < 0.001$)

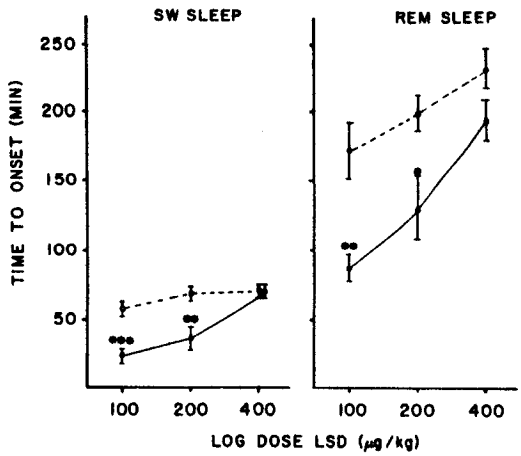

Fig. 3. Latencies to onset of SW sleep and REM sleep after the administration of LSD at the specified dosages to control rats (broken lines) and rats treated chronically with mescaline (solid lines). Both SW sleep and REM sleep onsets after the two lower doses of LSD occurred much earlier in the chronically mescaline-treated rats. (Each point represents the mean ± S.E.M. for 5 rats; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Discussion

The results of this study indicate the occurrence of some degree of tolerance to the central effects of mescaline in the rat. Tolerance to the stimulant effect of mescaline, as reflected by the latencies to SW sleep and REM sleep onset after the drug, developed very slowly and was still incomplete by the fifth day of chronic mescaline treatment. If the mescaline had been given longer prior to administration of test drugs, more tolerance would have probably developed.

The presence of cross tolerance to the arousal effect of mescaline in LSD and DET should indicate that these drugs may either share a common mode

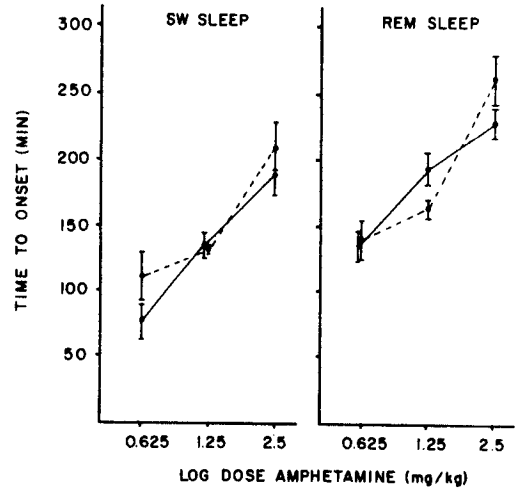

Fig. 4. Times to onset of SW sleep and REM sleep after the administration of amphetamine to control rats (broken lines) and rats treated chronically with mescaline (solid lines). The onsets of both SW sleep and REM sleep were similar in the 2 groups of animals. (Each point represents the mean ± S.E.M. for 5 rats)

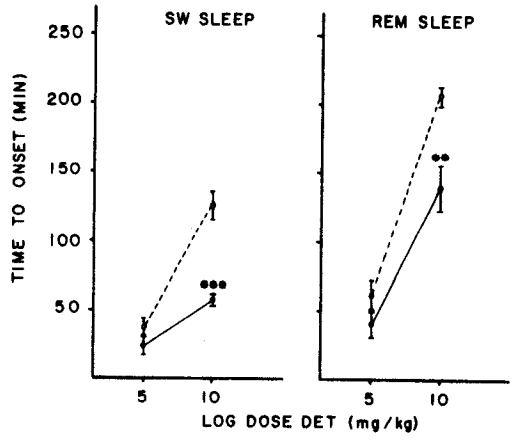

Fig. 5. Latencies to onset of SW sleep and REM sleep in control rats (broken lines) and rats treated chronically with mescaline (solid lines) in response to administration of DET. While these latencies were similar in both groups of animals after the lower DET dose, a significant shortening of both SW sleep and REM sleep onset for the mescaline-treated rats became apparent after the higher dose of DET. (Each point represents the mean ± S.E.M. for 5 rats; ** $P < 0.01$; *** $P < 0.001$)

of action or act on a final common pathway. The lack of cross tolerance in the case of amphetamine, on the other hand, suggests that this sympathomimetic owes its stimulant properties to a different mechanism. These results obtained with the EEG as an indicator of CNS function are thus in agreement with the findings of earlier studies on cross tolerance that have utilized behavioral parameters in the rat (Freedman *et al.*, 1958; Appel and Freedman, 1968) and physiological and behavioral parameters in man (Balestrieri and Fontanari, 1959; Wolbach *et al.*, 1962; Rosenberg *et al.*, 1963).

The finding of cross tolerance between mescaline and DET reported in this study and the presence of partial cross tolerance between LSD and DMT in man (Rosenberg *et al.*, 1964) should provide an indication that these hallucinogens may share a common mode of action. The demonstration of cross tolerance between LSD and tryptamine in chronic spinal dogs (Martin and Eades, 1972) adds support to this contention. The failure of tolerance to DMT to emerge in the EEG study of Gillin *et al.* (1973), on the other hand, may be partially related to the extremely short duration of action of this hallucinogen, a factor that would render the demonstration of tolerance to this agent particularly difficult.

Behavioral studies on mescaline have demonstrated that, while tolerance develops to the inhibitory, or disruptive, effects of the drug (Freedman *et al.*, 1958; Appel and Freedman, 1968), tolerance does not readily occur to the excitatory, or enhancing, effects (Bridger *et al.*, 1973). Tolerance to inhibitory effects of a drug on performance has been characterized as behavioral tolerance due to learning under the influence of the drug, as opposed to functional, or pharmacological, tolerance (Kalant *et al.*, 1971). A lack of tolerance to the rate-increasing effects of amphetamine has previously been reported by Schuster *et al.* (1966). This group consequently hypothesized that under circumstances where enhancing effects of drugs on performance emerge, tolerance would not be expected to develop.

The results of this report have demonstrated that the EEG provides a facile quantitative tool for studying tolerance and cross tolerance among hallucinogens. The usefulness of this method in testing the biological activity of chemical substances predicted on the basis of structure to be hallucinogenic has previously been postulated (Green *et al.*, 1973). The present work may further serve to demonstrate the applicability of the technique of continuous EEG and EMG recording during drug administration to the study of adaptive mechanisms to drugs affecting the central nervous system (Khazan, 1975).

Acknowledgements. The skillful technical assistance of Mr. Philip Cook in the conduct of this work is gratefully acknowledged.

References

Adey, W. R., Bell, F. F., Dennis, B. J.: Effects of LSD-25, psilocybin, and psilocin on temporal lobe EEG patterns and learned behavior in the cat. Neurology (Minneap.) **12**, 591–601 (1962)

Appel, J. B., Freedman, D. X.: Tolerance and cross tolerance among psychotomimetic drugs. Psychopharmacologia (Berl.) **13**, 267–274 (1968)

Balestrieri, A., Fontanari, D.: Acquired and crossed tolerance to mescaline, LSD-25 and BOL-148. Arch. gen. Psychiat. **1**, 279–282 (1959)

Bradley, P. B.: The effect of some drugs on the electrical activity of the brain in the cat. Electroenceph. clin. Neurophysiol. **5**, 471 (1953)

Bridger, W. H., Mandel, I. J., Stoff, D. M.: Mescaline: No tolerance to excitatory effects. Biol. Psychiat. **7**, 129–138 (1973)

Freedman, D. X., Aghajanian, G. K., Ornitz, E. M., Rosner, B. S.: Patterns of tolerance to lysergic acid diethylamide and mescaline in rats. Science **127**, 1173–1174 (1958)

Freedman, D. X., Appel, J. B., Molliver, M. E.: Tolerance to the behavioral effects of LSD-25 in rat. J. Pharmacol. exp. Ther. **143**, 309–313 (1964)

Gillin, J. C., Cannon, E., Magyar, R., Schwartz, M., Wyatt, R. J.: Failure of N,N-dimethyltryptamine to evoke tolerance in cats. Biol. Psychiat. **7**, 213–220 (1973)

Goldstein, L., Murphree, H. B., Pfeiffer, C. C.: Quantitative electroencephalography in man as a measure of CNS stimulation. Ann. N.Y. Acad. Sci. **107**, 1045–1056 (1963b)

Goldstein, L., Murphree, H. B., Sugarman, A. A., Pfeiffer, C. C., Jenney, E. H.: Quantitative electroencephalographic analysis of naturally occurring (schizophrenic) and drug-induced psychotic states in human males. Clin. Pharmacol. Ther. **4**, 10–21 (1963a)

Green, J. P., Dressler, K. P., Khazan, N.: Mescaline-like activity of 2-amino-7-hydroxytetralin. Life Sci. **12**, 475–479 (1973)

Hoch, P. H., Cattell, J. P., Pennes, H. H.: Effects of mescaline and lysergic acid (d-LSD-25). Amer. J. Psychiat. **108**, 579–584 (1952)

Kalant, H., LeBlanc, A. E., Gibbons, R. J.: Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol. Rev. **23**, 135–179 (1971)

Khazan, N.: Implications and significance of EEG and sleep-awake cycle in the study of experimental drug dependence. In: Methods in Narcotics Research, S. Ehrenpreis and A. Neidle, eds. New York: Dekker (in press, 1975)

Khazan, N., McCash, D.: Effects of LSD-25, N,N-dimethyltryptamine (DMT), and N,N-diethyltryptamine (DET) on the photic evoked responses in the unanesthetized rabbit. Arch. int. Pharmacodyn. **154**, 474–483 (1965)

Martin, W. R., Eades, C. G.: Cross tolerance to tryptamine in the LSD tolerant dog. Psychopharmacologia (Berl.) **27**, 93–98 (1972)

Munoz, C., Goldstein, L.: Influence of adrenergic blocking drugs upon the EEG analeptic effect of dl-amphetamine in conscious unrestrained rabbits. J. Pharmacol. exp. Ther. **132**, 354–359 (1961)

Pierre, R.: Effets sur l'EEG du lapin d'une intoxication chronique ou aigue par la diethylamide de l'acide lysergique (LSD-25). C.R. Soc. Biol. (Paris) **151**, 698–701 (1957)

Rosenberg, D. E., Isbell, H., Miner, E. J., Logan, C. R.: The effect of N,N-dimethyltryptamine in human subjects tolerant to lysergic acid diethylamide. Psychopharmacologia (Berl.) **5**, 217–226 (1964)

Rosenberg, D. E., Wolbach, A., Miner, E. J., Isbell, H.: Observations on direct and cross tolerance with LSD and d-amphetamine in man. Psychopharmacologia (Berl.) **5**, 1–15 (1963)

Schuster, C. R., Dockens, W. S., Woods, J. H.: Behavioral variables affecting the development of amphetamine tolerance. Psychopharmacologia (Berl.) **9**, 170–182 (1966)

Smythies, J. R., Sykes, E. A.: The effect of mescaline upon the conditioned avoidance response in the rat. Psychopharmacologia (Berl.) **6**, 163–172 (1964)

Weeks, J. R.: Long-term intravenous infusion. In: Methods in Psychobiology, vol. 2, R. O. Myers, ed., pp. 155–168. New York: Academic Press 1972

Wolbach, A. B., Isbell, H., Miner, E. J.: Cross tolerance between mescaline and LSD-25 with a comparison of the mescaline and LSD reactions. Psychopharmacologia (Berl.) **3**, 1–14 (1962)

Dr. Naim Khazan, University of Maryland School of Pharmacy
636 W. Lombard Street, Baltimore, Md. 21201, U.S.A.