PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 109–113 (1974)

Marijuana Responses in Rats: Influence of Castration or Testosterone[1] (38053)

R. A. COHN, E. S. BARRATT, AND J. H. PIRCH

Department of Pharmacology and Toxicology and Department of Neurology and Psychiatry, University of Texas Medical Branch, Galveston, Texas 77550

In a previous study, we demonstrated a sex difference in the responses of rats to marijuana extract by means of a quantitative behavior rating technique (1). Female rats were found to have significantly greater responses than males to oral doses of Δ^9-THC of 5, 10, 20 and 40 mg/kg.

Sex differences in the responses of rats to other drugs have been known for some time (2). Female rats show greater responses than male rats to hexobarbital (3), strychnine (4), nicotine (5), picrotoxin (6), aminopyrine (7), and morphine (8).

Axelrod, studying the *N*-demethylation of narcotic drugs, demonstrated that liver microsomal enzyme activity is greater in male than in female rats (8). The sex difference in the responses of rats to drugs is in many cases attributed to this difference in liver microsomal enzyme activity; male rats metabolize many drugs more rapidly than females and thus show less effect.

Booth and Gillette have presented evidence that the level of drug metabolizing activity is related to the anabolic effects of testosterone (9). Other investigators have shown that castration decreases the liver microsomal enzyme activity and increases the responses of male rats to drugs; in female rats, testosterone pretreatment increases the microsomal enzyme activity and decreases the responses to drugs (3, 7, 10). The present study was aimed at examining: (1) the effects of alteration of hormonal balance on responses of rats to marijuana and (2) the effects of liver microsomal enzyme induction and inhibition on responses to marijuana.

Materials and Methods. Male and female albino rats (Sprague-Dawley descent) were obtained from Texas Inbred Mice Co., Houston, Texas. Animals ranged in weight from 200–300 g at the time of testing. In experiments involving both male and female rats, weights of animals were matched to within 20 g.

Marijuana extract containing 17.1% Δ^9-THC, 1.7% cannabidiol, 5.4% cannabinol and other undetermined cannabinoids was generously supplied by Dr. J. A. Scigliano, NIMH. The extract was diluted to a concentration of 20 mg/ml Δ^9-THC with 6% Tween 80 in distilled water. Placebo consisted of the 6% Tween 80 suspending medium. The diluted suspension was administered through a polyethylene tube (PE 90) inserted into the mouth of the rat and passed 10–12 cm down the esophagus to the stomach. In all experiments, the extract was administered at a dose of Δ^9-THC of 20 mg/kg. The volume of placebo administered was 1 ml/kg.

Testosterone propionate (Oreton, Schering) was administered subcutaneously. Sodium pentobarbital (Nembutal, Abbot Laboratories), sodium phenobarbital (USP,

[1] This paper represents a portion of the research in a Dissertation submitted by R. A. C. in partial fulfillment of requirements for the Ph.D. degree. This research was supported in part by U. S. Public Health Service research grant DA 00870 and in part by a grant from the U. S. Department of Justice. The fact that the National Institute of Law Enforcement and Criminal Justice furnished financial support to the activity described in this publication does not necessarily indicate the concurrence of the Institute in the statements or conclusions contained herein.

Merck), and SKF 525A (2-diethylaminoethyl-2, 2-diphenylvalerate hydrochloride) were administered intraperitoneally.

The behavior rating technique was described previously (1). Animals were tested in a series of quantified behavioral tasks in which increasing scores reflect increased levels of intoxication. Tasks included: 1) the length of time an animal remained on a platform, 2) time on a bar suspended 3 in. from a table top, 3) time on a bar elevated 4 feet from the floor, 4) vocalization in response to handling, 5) response to presentation of a wooden pencil, and 6) response to being dropped from a height of 1 ft.

In order to test the effect of castration on marijuana responses, 24 male weanling rats were castrated under ether anesthesia; an additional 24 male weanling rats received sham operations. Thirty days after surgery half of the animals from each group were treated with marijuana extract; the remainder received placebo. Three hours later, animals were tested in the behavioral tasks.

The influence of testosterone on responses to marijuana was examined by pretreating 24 female rats with 3.8 mg testosterone propionate every other day for 30 days. An additional 24 females received peanut oil every other day for 30 days. Half of the animals from each group were administered marijuana extract; the remainder received placebo. Three hours later, animals were tested in the behavioral tasks.

In order to induce the liver microsomal enzyme system, 15 female rats were treated daily for 3 days with 75 mg/kg sodium phenobarbital divided into two doses (11). Fifteen control rats were treated with saline. Twenty-four hours after the last phenobarbital injection, 10 animals from each group were administered marijuana extract, the remaining 5 rats in each group received placebo. All animals were tested in the behavior rating tasks 3 hr after drug administration.

Inhibition of the liver microsomal enzyme system was accomplished by pretreating 14 male rats with SKF 525A in a dose of 25 mg/kg (12). Fourteen control rats were pretreated with saline. Thirty minutes after pretreatment, 10 animals from each group were administered marijuana extract; the remaining 4 rats in each group received placebo. One hour after marijuana or placebo administration, all animals were tested in the behavioral tasks.

Statistical comparisons of behavior rating scores were made by means of the two-tailed Mann-Whitney U test while sleeping times were compared by the t test for grouped data.

Results. Orchiectomy had a significant effect on the responses of male rats to marijuana extract (Fig. 1a). Statistical analysis indicated that responses were significantly greater in castrated than sham-operated animals ($P < 0.002$). Responses of the castrated rats and of the sham-operated rats after treatment with placebo did not differ ($P > 0.10$). Marijuana, when compared to placebo, had a significant effect on both castrated ($P < 0.002$) and sham-operated ($P < 0.002$) animals.

Testosterone pretreatment had a significant effect on the responses of female rats to marijuana (Fig. 1b). Rating scores after marijuana were significantly higher in peanut oil pretreated females than in testosterone pretreated females ($P < 0.002$). There was no significant difference between the responses of testosterone pretreated and peanut oil pretreated animals to placebo ($P > 0.10$). Marijuana, when compared to placebo, had a significant effect on both testosterone pretreated ($P < 0.002$) and peanut oil pretreated ($P < 0.002$) animals.

Behavior rating scores of phenobarbital pretreated rats after marijuana did not differ significantly ($P > 0.10$) from saline pretreated animals (Fig. 2). In order to insure that this schedule of phenobarbital treatment successfully induced the liver microsomal enzyme system, pentobarbital sleeping time was assayed. Mean sleeping time after pentobarbital (35 mg/kg, ip) was significantly less ($P < 0.001$) in phenobarbital pretreated (18 ± 1 min) than in saline pretreated rats (119 ± 10 min) with 7 animals per group.

Behavior rating scores of SKF 525A pretreated rats after marijuana were significantly greater ($P < .02$) than scores of

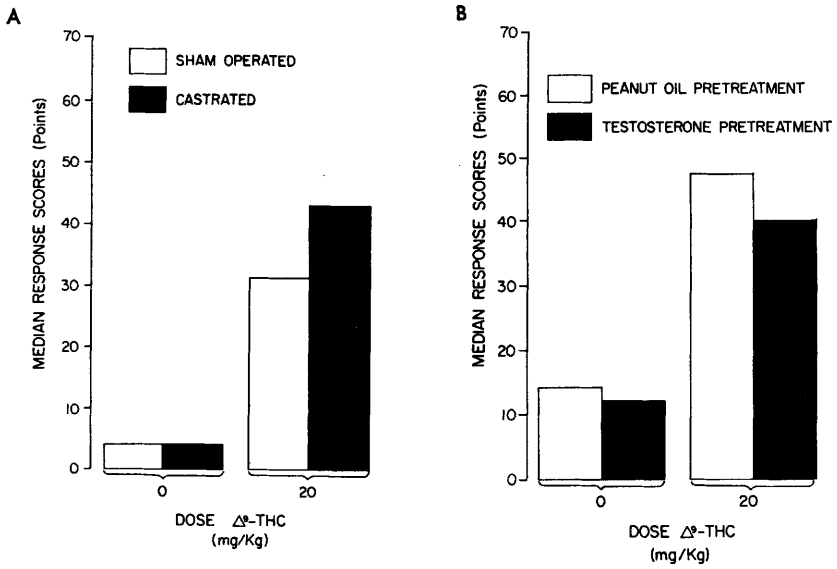

FIG. 1. Effects of alteration of hormonal balance on responses to marijuana: (a) castration; (b) testosterone pretreatment.

saline pretreated animals (Fig. 3). SKF 525A pretreatment did not affect the response of animals to placebo. In order to insure that this dose of SKF 525A successfully inhibited the liver microsomal enzyme system, pentobarbital sleeping time was assayed. Sleeping time after pentobarbital (40 mg/kg ip) was significantly greater ($P < 0.01$) in SKF 525A pretreated animals (259 ± 46 min) than in saline control rats (76 ± 9 min) with 5 animals per group.

Discussion. Several investigators have presented evidence that enzyme induction by phenobarbital alters the metabolism of Δ^9-THC. Nakazawa and Costa (13) found that liver homogenates of rats pretreated with phenobarbital metabolized Δ^9-THC at a rate twice that of liver homogenates from untreated rats. Cohen and coworkers (14) presented data showing that the magnitude of the cytochrome P_{450} difference spectrum to Δ^9-THC was greater in liver microsomes obtained from phenobarbital treated rats than in those obtained from untreated rats.

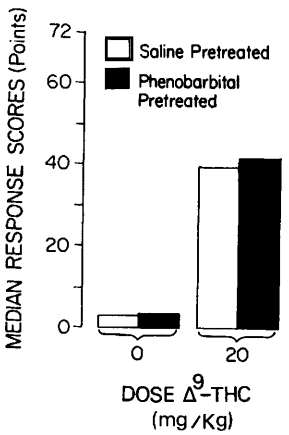

FIG. 2. Effect of liver microsomal enzyme induction on responses to marijuana.

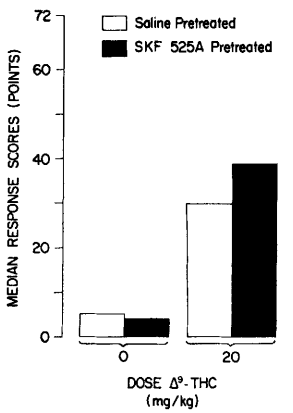

FIG. 3. Effect of liver microsomal enzyme inhibition on responses to marijuana.

Thus, while phenobarbital pretreatment probably increased the rate of metabolism of Δ^9-THC in our experiments, it failed to alter the behavioral responses to marijuana.

On the other hand, SKF 525A increased the behavioral effects of marijuana in male rats. This is in accord with the work of Sofia and Barry (15) who found that SKF 525A increased the Δ^1-THC[2] enhancement of barbital sleeping time. Since the duration of action of barbital is independent of liver metabolism, Sofia and Barry concluded that SKF 525A produced its effect by inhibiting the metabolism of Δ^1-THC.

Responses of males to marijuana were increased by castration while responses of females to marijuana were decreased by testosterone pretreatment. The normally occurring sex difference in liver microsomal enzyme activity is generally attributed to the anabolic effects of testosterone (10). Whether the observed sex difference in the response of rats to marijuana is related to the anabolic effects of testosterone on metabolism remains to be determined.

We are not the only group to demonstrate a sex difference in the responses of rats to marijuana. Park and Tilton (16) found sex differences in the conditioned avoidance responses of rats treated with marijuana smoke. Thompson and coworkers (17), conducting chronic oral toxicity studies, found a consistently higher incidence of mortality in female than in male rats treated with crude marijuana extract, Δ^9-THC, or Δ^8-THC. Recently, Borgen and coworkers (18) demonstrated that female rats show a greater hypothermic response than male rats to synthetic Δ^9-THC or crude marijuana extract.

The mechanism of this sex difference has not been determined. One cannot question the role of the liver microsomal enzyme system in the biotransformation of Δ^9-THC, but this may not be the complete answer. Recently, Burstein and Kupfer (19) reported that the rate of hydroxylation of Δ^1-THC is greater with liver microsomes from male rats than with microsomes from female rats. If this male–female difference in hydroxylation plays an important role in the sex difference in behavior, then both induction and inhibition of the microsomal enzyme involved in this reaction should influence behavior responses to marijuana. Pretreatment with SKF 525A has been shown to inhibit the hydroxylation of Δ^1-THC (20) while pretreatment with phenobarbital has been reported to induce hydroxylation of Δ^9-THC (13). Our finding that phenobarbital pretreatment did not alter behavior responses to marijuana suggests that the sex difference in behavior may, in part, be due to factors other than the rate of hydroxylation of Δ^9-THC.

Since 11-hydroxy-Δ^9-THC has been shown to be as active as Δ^9-THC (21), phenobarbital pretreatment may only serve to enhance the conversion of one active compound to another active compound. It is possible that later steps in the biotransformation of Δ^9-THC may be more important for termination of behavioral effects. Although SKF 525A has been reported to inhibit the further metabolism of 11-hydroxy-Δ^9-THC (20), there are no reports concerning the effects of phenobarbital on the metabolism of 11-hydroxy-Δ^9-THC, nor are there studies on sex differences in metabolism of this compound.

The sex difference in behavior may not be entirely related to the liver metabolism of Δ^9-THC. Nakazawa and Costa (13) have demonstrated in rats that the lung may be important for the metabolism of Δ^9-THC, that 3-methylcholanthrene can induce the enzyme system responsible for the metabolism of Δ^9-THC in the lung but not in the liver, and that the pathways of Δ^9-THC metabolism in the lung may be different from those in the liver. It is possible that the sex difference in behavior is the result of a sex difference in the metabolism of Δ^9-THC by the lung.

Alternatively, the sex difference in behavior may be unrelated to the metabolism of the drug. While the rat and mouse are believed to be the only species in which a significant sex difference in the drug metabolizing system is present (5, 22), some investigators have alluded to the presence of a sex difference in response to marijuana in

[2] Δ^1-THC = Δ^9-THC.

rhesus monkeys (23) and man (24). It would be of importance to confirm the sex differences in these species and to elucidate the mechanisms of these differences.

Summary. Female rats show a significantly greater behavioral response than male rats to marijuana extract. This difference in response was partially abolished through castration of males or testosterone pretreatment of females. Since castration and testosterone pretreatment may affect drug metabolism, we also studied the effects of liver microsomal enzyme induction and inhibition on responses of rats to marijuana. While enzyme inhibition increased the responses of rats to marijuana, enzyme induction did not alter behavioral responses to marijuana.

1. Cohn, R. A., Barratt, E., and Pirch, J. H., Proc. Soc. Exp. Biol. Med. **140**, 1136 (1972).

2. Nicholas, J. S., and Barron, D. H., J. Pharmacol. Exp. Ther. **46**, 125 (1932).

3. Quinn, G. P., Axelrod, J., and Brodie, B. B., Biochem. Pharmacol. **1**, 152 (1958).

4. Poe, C. F., Suchy, J. F., and Witt, N. E., J. Pharmacol. Exp. Ther. **58**, 239 (1936).

5. Holck, H. G. O., Munis, A. K., Mills, L. M., and Smith, E. L., J. Pharmacol. Exp. Ther. **60**, 323 (1937).

6. Holck, H. G. O., J. Amer. Pharmaceut. Ass. **38**, 604 (1949).

7. Kato, R., and Gillette, J. R., J. Pharmacol. Exp. Ther. **150**, 279 (1965).

8. Axelrod, J., J. Pharmacol. Exp. Ther. **117**, 322 (1956).

9. Booth, J., and Gillette, J. R., J. Pharmacol. Exp. Ther. **137**, 374 (1962).

10. Murphy, S. D., and DuBois, K. P., J. Pharmacol. Exp. Ther. **124**, 194 (1958).

11. Conney, A. H., Davidson, C., Gastel, R., and Burns, J. J., J. Pharmacol. Exp. Ther. **130**, 1 (1960).

12. Gillette, J. R., Prog. in Drug Res. **6**, 11 (1963).

13. Nakazawa, K., and Costa, E., Nature **234**, 48 (1971).

14. Cohen, G. M., Peterson, D. W., and Mannering, G. J., Life Sci. **10**, Part 1, 1207 (1971).

15. Sofia, R. D., and Barry III, H., Eur. J. Pharmacol. **13**, 134 (1970).

16. Park, Y. Y., and Tilton, B. E., Proc. West. Pharmacol. Soc. **13**, 151 (1970).

17. Thompson, G. R., Rosenkrantz, H., Schoeppi, U. H., and Braude, M. C., Toxicol. Appl. Pharmacol. **25**, 363 (1973).

18. Borgen, L. A., Lott, G. C., and Davis, W. M., Res. Comm. Chem. Pathol. Pharmacol. **5**, 621 (1973).

19. Burstein, S., and Kupfer, D., Ann. N. Y. Acad. Sci. **191**, 61 (1971).

20. Truitt, E., Fifth International Congress on Pharmacology: Abstracts of Volunteer Papers, p. 236, #1416 (1972).

21. Christensen, H. D., Freudenthal, R. I., Gidley, J. T., Rosenfeld, R., Boegli, G., Testino, L., Brine, D. R., Pitt, C. G., and Wall, M. E., Science **172**, 165 (1971).

22. Schenkman, J. B., Frey, I., Remmer, H., and Estabrook, R. W., Mol. Pharmacol. **3**, 517 (1967).

23. Dr. R. Mechoulam in Discussions of a paper by Burstein, S., and Kupfer, D., Ann. N. Y. Acad. Sci. **191**, 66 (1971).

24. Tart, C., On Being Stoned, Science and Behavior Books, Palo Alto (1971).

Received Nov. 19, 1973. P.S.E.B.M., 1974, Vol. 146.