Pharmacology Biochemistry & Behavior, Vol. 6, pp. 183–185.

Tolerance to the Effects of Δ⁹-THC on Shuttle-Box Performance and Body Temperature

M. TEN HAM

Laboratory of Pharmacology, National Institute of Public Health
P.O. Box 1, Bilthoven 2660, The Netherlands

(Received 6 January 1976)

TEN HAM, M. *Tolerance to the effects of Δ⁹-THC on shuttle-box performance and body temperature in rats.* PHARMAC. BIOCHEM. BEHAV. 6(2) 183–185, 1977. – Two groups of rats were trained in a shuttle-box and received Δ⁹-tetrahydrocannabinol (Δ⁹-THC), either before or after being tested. The drug-before group showed tolerance – within 3–6 sessions – to the response-inhibiting effect of THC. The drug-after animals appeared also to be tolerant when they received Δ⁹-THC before being tested. It is concluded that the tolerance to this effect probably is not learned, but has a physiological base. This is corroborated by the finding that during the same study all the animals developed tolerance to the hypothermic effect of Δ⁹-THC.

Learned tolerance Behavior Body temperature Δ⁹-Tetrahydrocannabinol

MANY authors studied the phenomenon of tolerance to marihuana effects. Most of the them showed development of tolerance after a few administrations, e.g. disruption of pigeon and rat behavior in a conditioned task [11,12] or hypothermia in rats [9] and in mice [5].

Several mechanisms for this tolerance have been suggested. One possibility is induction of the microsomal liver enzymes responsible for the biotransformation of marihuana, but the literature on the effect on biotransformation enzymes is not very consistent.

Another hypothesis proposes that tolerance develops not through a relatively simple physiological process, such as enzyme induction, but because during the experiments the experimental animal learns in a more complicated manner to compensate for any changes in its environment. It has been shown that such a mechanism, which is called learned tolerance, at least partially explains tolerance to certain effects in rats [1, 2, 7, 15] and in monkeys [10].

Carder and Olson [2] tried to discriminate between the two alternatives with the method of Chen [3]. In this study one group of animals (DB, drug before) received THC-treatment before testing in a bar-press situation for food or water reward, a second group after being tested (DA, drug after).

In our study we conducted two analogous experiments and compared the results with simultaneously obtained data of the body temperature of the same rats. If it could be shown that two mechanisms for the development of tolerance exist in the same animal, many seemingly contradictory results would be explained.

METHOD

Thirty-two male Wistar rats, supplied by Centraal Proefdierenbedrijf TNO, Zeist, were trained in a standard Lehigh Valley shuttle-box in a sound-isolated chamber with random noise to a performance of 16 correct responses out of 20 trials. Each trial consisted of 35 sec intertrial interval, six seconds conditioned stimulus (light) and an unconditioned stimulus (electric shock), which was terminated by the animal running to the opposite site, or after maximum 10 sec.

After a stable baseline had been established, the animals were divided at random into two groups, DB (drug before) and DA (drug after). For three days both groups received 1 ml of the vehicle (4% Tween-80 in saline) intraperitoneally 1 hr before and 15 min after testing. During the next days group DB received 20 mg/kg Δ⁹-tetrahydrocannabinol (THC, batch UNC 441) 1 hr before testing, group DA the same 15 min after testing.

Body temperature was determined in all animals 1 hr after each injection. THC-treatment according to DB or DA was continued until tolerance had developed to the hypothermia, which we considered to be complete when on three consecutive days the mean temperature differed by not more than 0.5°C from the average of the pretreatment period.

When the animals had become tolerant to hypothermia, the DA group was shifted to a drug before scheme, and received its treatment 1 hr before testing. Two experiments were carried out; in the first one the animals were treated and tested every day, in the second experiment every other day to prevent effects caused by cumulation, since THC is still present in the body after 24 hr [8].

Differences were tested for statistical significance (0.05 level), either parameter free with Mann-Whitney's U-test (shuttle-box performance), or with analysis of variance (body temperature).

RESULTS

Figure 1 presents the data of the shuttle-box performance in the first experiment. Quite clearly the DB-group develops tolerance in two or three days of treatment. The DA shows a slight decline in number of responses, but stays virtually on the same level for the next four days. On Day 9 (test) the DA was reversed and it appeared that these animals were all tolerant to the disrupting effect of THC.

The body temperatures are depicted in Fig. 2. Most animals reached the tolerance criterion after three days of treatment, some after four days. No hypothermia is seen in the DA before testing (this means: 1 hr after saline injection, or 23 hr after THC). Differences in shuttle-box performance were statistically significant only on the first day of treatment, body temperatures on the first two days of treatment.

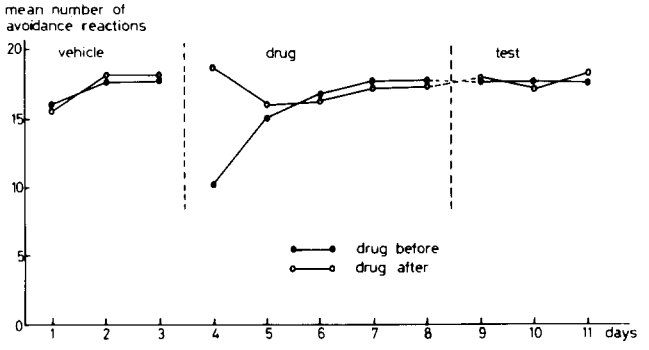

FIG. 1. Tolerance to the disrupting effect of THC on shuttle-box performance. THC (20 mg/kg) was given IP 1 hr before (drug before) or 15 min after drug after) testing.

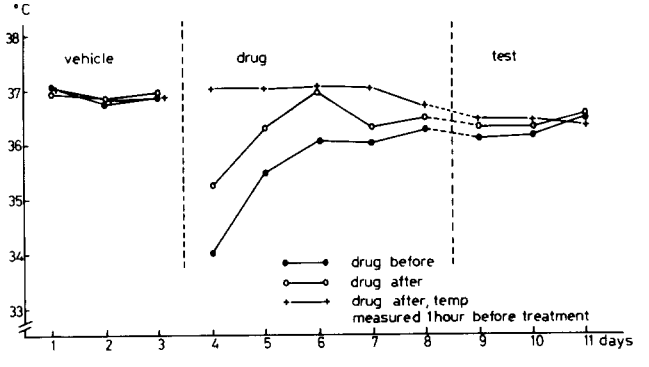

FIG. 2. Tolerance to the hypothermic effect of THC. Rectal body temperature was measured 1 hr before treatment (drug after, +——+) or 1 hr after treatment (drug before ●——●, and drug after ○——○) THC 20 mg/kg was administered IP daily.

Figures 3 and 4 represent results of the second experiment. Except that development of tolerance in the DA takes many more days than in DB, the pattern of response is the same: on the test day the DA shows the same tolerance when treated before testing as the DB does. Due to a misunderstanding, the DB-group was not treated on the day the DA group was tested, but as tolerance to the shuttle-box disrupting effect as well as to the hypothermic effect was present on Day 19, this hardly effects the

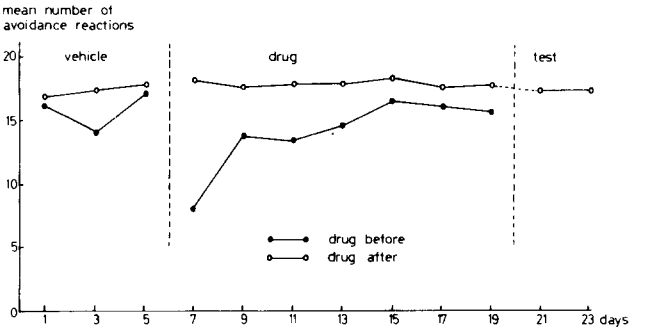

FIG. 3. Tolerance to the disrupting effect of THC on shuttle-box performance. THC (20 mg/kg) was given IP 1 hr before (drug before) or 15 min after (drug after) testing.

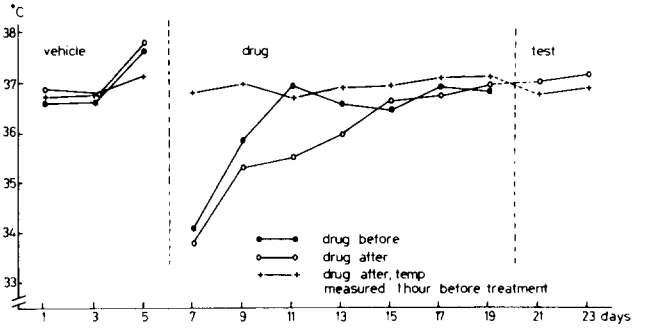

FIG. 4. Tolerance to the hypothermic effect of THC. Rectal body temperature was measured 1 hr before treatment (drug after, +——+) or 1 hr after treatment (drug before ●—— ——● and drug after ○——○) THC 20 mg/kg was administered IP daily.

conclusions which can be drawn from this experiment. The differences in shuttle-box performance in this experiment were significant on the first four days of treatment, the body temperatures on the first three days.

During the experiments the animals vocalized when handled and showed the so-called popcorn response. This behavior lasted three to four days during the experiment.

DISCUSSION AND CONCLUSION

Our results do not indicate a learning process in the development of tolerance to the THC-effect in a shuttle-box performance. Some explanations for the disagreement with the Carder and the Manning studies are possible.

One is species difference in the experimental animals. This holds for Manning's monkey experiments, but it is hardly possible to explain the discrepancy with Carder's results this way. More than likely it is an influence of the nature of the experiment. Carder worked with a different task: food or water reward.

The action of THC on bar-pressing behavior in Carder's experiment could therefore be twofold: directly by the sedative action, and indirectly via the de-appetizing activity of THC. The test day is the first day for the DA-group to respond in drugged condition and even if his animals have developed biochemical tolerance to the sedative action, the effect of THC can still be just strong enough to disturb bar pressing if there is no tolerance to the influence of THC on

food intake. In the shuttle-box behavior we have only the direct sedative influence of THC, and this activity of THC after a period of treatment is perhaps not strong enough to disrupt the animal's behavior. This is even more likely since it is known that behavior maintained by food reinforcement is susceptible to changes in physiological mechanisms not important to behavior maintained by shock avoidance [14].

Our results further agree with the findings of Johansson, Henriksson and Järbe [7], who noticed that THC injected before the test, did not disrupt the avoidance response of rats previously treated with THC two hours after each training session. Rats treated with vehicle for eight days showed a strong THC-effect when tested in a drug-before situation.

Tolerance to the sedative effect is probable, because the rat's vocalizing when touched, which seems closely linked to the sedative action [6] no longer occurred after about four days of treatment.

A further possibility is a role of the THC metabolism. Some enzyme induction may occur, leading to a more rapid disappearance of THC and/or active metabolities. This is not unlikely, since Sofia and Barry [15] have shown that SKF-525, a well-known inhibitor of microsomal enzymes, enhances THC-activity. The rapidly developing tolerance for the hypothermic effect, as displayed in Figs. 2 and 4, might be explained by enzyme induction without assuming any learning effect. The same holds for the shuttle-box tolerance. But, there are literature reports [5,13] indicating that THC is a enzyme blocker itself – which is in contrast with Sofia and Barry's findings [15] – and this would result in an increased amount of THC and a decreased amount of metabolites. If so, tolerance to the hypothermia and shuttle-box tolerance could only be explained by assuming that this effect is caused by THC-metabolites.

Evaluation of these explanations will require more information about the levels of THC and its metabolites in blood and brain tissue, and also about the pharmacological activity of these metabolites in comparison with THC.

Further a certain tissue or cell tolerance cannot be excluded, although mechanisms in such a process are much more complex and less easily accessible. Finally, a combination of some or all of these factors is possible.

ACKNOWLEDGEMENT

The author is indebted to Mr. W. van Veenendaal for his careful technical assistance.

REFERENCES

1. Bueno, O. F. A. and E. A. Carlini. Dissociation of learning in Marihuana tolerant rats. *Psychopharmacologia* **25**: 49–56, 1972.
2. Carder, B. and J. Olson. Learned behavioral tolerance to marihuana in rats. *Pharmac. Biochem. Behav.* **1**: 73–76, 1973.
3. Chen, C. S. A study of the alcohol-tolerance effect and an introduction of a new behavioral technique. *Psychopharmacologia* **12**: 433–440, 1968.
4. Ham, M. ten, Engelina M. den Tonkeliar and J. M. Koomen. Influence of two tetrahydrocannabinols (THC's) on the activity of biotransformation enzymes. In: *Alcohol Intoxication and Withdrawal*, edited by M. Gross. New York: Plenum Press, 1973.
5. Ham, M. ten and Y. de Jong. Tolerance to the hypothermic and aggression-attenuating effect of Δ^8- and Δ^9-tetrahydrocannabinol in mice. *Eur. J. Pharmac.* **28**: 144–148, 1974.
6. Henriksson, B. G. and T. U. C. Järbe. Cannabis-induced vocalization in the rat. *J. Pharm. Pharmac.* **23**: 457–458, 1971.
7. Johansson, J. O., B. G. Henriksson and T. U. C. Järbe. Effects of Δ^8-THC on dissociation of conditioned avoidance responding in tolerant and nontolerant rats. *Physiol. Psychol.* **2**: 431–432, 1974.
8. Klausner, H. H. and J. V. Dingell. The metabolism and excretion of Δ^9-tetrahydrocannabinol in the rat. *Life Sci.* **10**: 49–59, 1971.
9. Lomax, P. Acute tolerance to the hypothermic effect of marihuana in the rat. *Res. Communs chem. pathol. Pharmac.* **2**: 159–167, 1971.
10. Manning, F. J. Acute tolerance to the effect of delta-9-tetrahydrocannabinol on spaced responding by monkeys. *Pharmac. Biochem. Behav.* **1**: 665–671, 1973.
11. Masur, J., R. M. W. Märtz and E. A. Carlini. Effects of acute and chronic administration of Cannabis sativa and (-)-Δ^9-tetrahydrocannabinol on the behaviour of rats in an open field arena. *Psychopharmacologia* **19**: 388–397, 1971.
12. McMillan, D. E., L. S. Harris, J. M. Frankenheim and J. S. Kennedy. Development of marked behavioral tolerance to l-Δ^8-tetrahydrocannabinol (Δ^8-THC) in the pigeon. *Pharmacologist* **12**: 322, 1970.
13. Paton, W. D. M. and R. G. Pertwee. Effects of cannabis and certain of its constitutents on pentobarbitone sleeping time and phenazone metabolism. *Br. J. Pharmac.* **44**: 250–261, 1972.
14. Sidman et al., cited by Harris, R. T., W. Waters and D. McLendon. Behavioral effects in Rhesus monkeys of repeated doses of Δ^9-tetrahydrocannabinol. *Psychopharmacologia* **26**: 297–306, 1972.
15. Sofia, R. D. and H. Barry III. Depressant effect of Δ^1-tetrahydrocannabinol enhanced by inhibition of its metabolism. *Eur. J. Pharmac.* **13**: 134–137, 1970.