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TOXICOLOGY OF MARIHUANA

ROBERT B. FORNEY

Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana

Marihuana is an ill-defined conglomerate of chemicals. Only recently has an index of its potency been defined in terms of its Δ^9-tetrahydrocannabinol (Δ^9-THC) content. Although Δ^9-THC appears to be responsible for the principle toxicological effect, at this point one can scarcely assume that marihuana, or even an extract of marihuana, and Δ^9-THC are synonymous toxicologically. In an effort to minimize confusion, this paper will be confined to animal studies with 97 to 99% pure Δ^9-THC derived in most cases from Thailand marihuana.

Phillips *et al.* (11) have investigated the acute toxicity of pure Δ^9-THC in male albino Holtzman rats weighing 100 to 125 g and in male albino Cox mice weighing 20 to 25 g. The compound was dissolved in 10% Tween 80 and was administered intravenously, intraperatoneally and intragastrically. The data obtained with rats are presented in table 1. The LD50 values in mg/kg are: intravenous 28.6, intraperatoneal 373 and intragastric 666. The data obtained with mice are presented in table 2. The LD50 values in mg/kg are: intravenous 42.4, intraperatoneal 455 and intragastric 481. After having received an intravenous injection of Δ^9-THC both the rats and mice became ataxic in 1 to 2 min. If stimulated by either touch or sound, they became hyperactive for 1 to 2 sec. Depression appeared to increase in these animals although their sensitivity to stimulation remained for about 1 hr. The righting reflex was lost and dyspnea progressed to apnea in these animals. Death occurred with respiratory depression. Postmortem examination revealed that the lungs of all animals were both congested and edematous. All other organs were unremarkable. Survivors were free of toxic signs after 24 to 72 hr.

Phillips *et al.* (10) have also administered water suspended Δ^9-THC to rats intraperitoneally each day for 30 days. Peritoneal injection was made at six different sites in each animal to minimize tissue reaction and to maximize absorption. Five dose levels ranging from 0 to 30 mg/kg were used. On the 30th day, the animals continued to be hypersensitive to external stimuli for approximately 0.5 hr after injection. This period was followed by increasing ataxia, lacrimation, diarrhea and depression. During the final 10 days of the experiment, all the post-injection symptoms were exaggerated over those of the first 10 days but were qualitatively the same. There was no evidence of developing tolerance, but rather, of increasing sensitivity. In the chronic studies, a gross examination of the peritoneal injection sites was made post mortem. A significant tissue reaction was not observed and no evidence of incomplete absorption of the compound was seen.

Carlini (2) has reported that rats can develop tolerance to the behavioral effects of THC when low doses are administered over short periods of time.

King and Forney (4), in 1966, found relatively high concentrations of Δ^9-THC

TABLE 1

Acute toxicity of Δ^9-tetrahydrocannabinol

(Animal, rat; vehicle, 10% Tween 80)

Route of Administration	No. Animals/Group	Observation Time	LD50*
		days	*mg/kg*
I.v.	6	7	28.6 (27.4–29.9)†
I.p.	6	7	373 (305–454)
I.g.	6	7	666 (604–734)

* Method of Weil (14).

† Confidence interval 95%.

TABLE 2

Acute toxicity of Δ^9-tetrahydrocannabinol

(Animal, mouse; vehicle, 10% Tween 80)

Route of Administration	No. of Animals/Group	Observation Time	LD50*
		days	*mg/kg*
I.v	6	7	42.5 (36.7–48.9)†
I.p.	10	7	455 (419–493)
I.g.	10	7	482 (451–515)

* Method of Weil (14).

† Confidence interval 95%.

in the adipose tissue of rats 24 hr after intragastric administration of 100 mg of THC. Agurell *et al.* (1) have reported similar findings in rabbits 3 days after administration of 0.6 mg/kg of ^{14}C Δ^9-THC.

Turk (13) gave 100 mg/kg of Δ^9-THC suspended in 10% Tween 80 intragastrically and analyzed selected tissues at 3, 6, 12 and 24 hr for Δ^9-THC content. At 3 hr, THC was identified in blood, brain, heart, kidney, liver, lung and spleen, but not in epididymal fat pads. At 6 hr, the liver concentration of THC was markedly reduced and the other tissues had somewhat lower concentrations. At 12 hr, Δ^9-THC could no longer be found in brain, heart or liver, but was found in epididymal fat pads. At 24 hr, only kidney, lung and epididymal fat retained Δ^9-THC.

Phillips *et al.* (9) have studied the effects of Δ^9-THC in combination with hexobarbital or ethanol in male albino Harlan rats weighing from 100 to 125 g. Groups of eight rats were given intraperitoneally either 10, 20 or 40 mg/kg of Δ^9-THC as a buffered water suspension alone or followed by an intraperitoneal injection of hexobarbital, 100 mg/kg, or followed by an intraperitoneal injection

of 1, 2 or 3 g/kg of ethanol. The dose range of Δ^9-THC was expanded to include 2.5 and 5.0 mg/kg when 2.0 g/kg of ethanol were given and further to 1.25 mg/kg when 3.0 g/kg of ethanol were given. The effect on sleeping time and immobility time was measured. Immobility time is defined as the interval between the loss of the righting reflex and the time the animals were able to leave a 6-inch circle. The data obtained with the combination of Δ^9-THC and hexobarbital are illustrated in figure 1. None of animals slept or were immobile with Δ^9-THC alone, but slept an average of 22 min with hexobarbital alone. Sleeping and immobility times of hexobarbital were enhanced in a dose related fashion when Δ^9-THC was also given. A similar pattern was demonstrated with ethanol as shown in figures 2 and 3. The effects of Δ^9-THC in combination with hexobarbital appeared to be additive. Animals receiving 1 or 2 g/kg of ethanol alone did not sleep, but in combination with even 2.5 mg/kg of Δ^9-THC they appeared to be more depressed. When 3.0 g/kg of ethanol were given, 4 of 8 rats that received 1.25 mg/kg of Δ^9-THC slept. In combination with Δ^9-THC, ethanol depression was greatly augmented. This enhancement was not as striking when doses of Δ^9-THC exceeding 5 or 10 mg/kg were given. This may suggest that receptor sites are rapidly saturated and when the dose exceeded 5 or 10 mg/kg, the excess Δ^9-THC was eliminated or taken up by lipoid tissue. It is also possible that with the higher doses of Δ^9-THC, the stimulant effect of Δ^9-THC more effectively antagonized the ethanol.

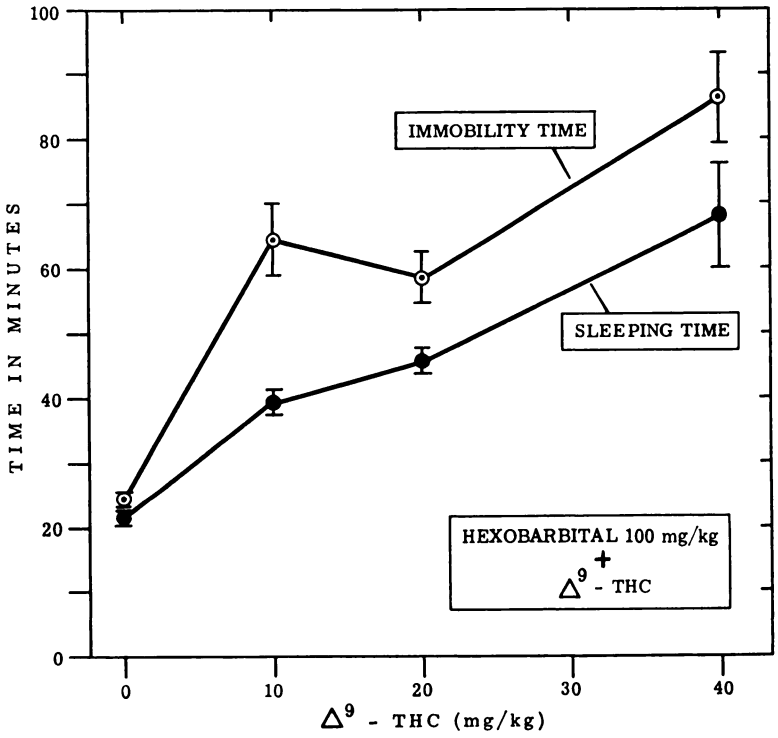

FIG. 1.

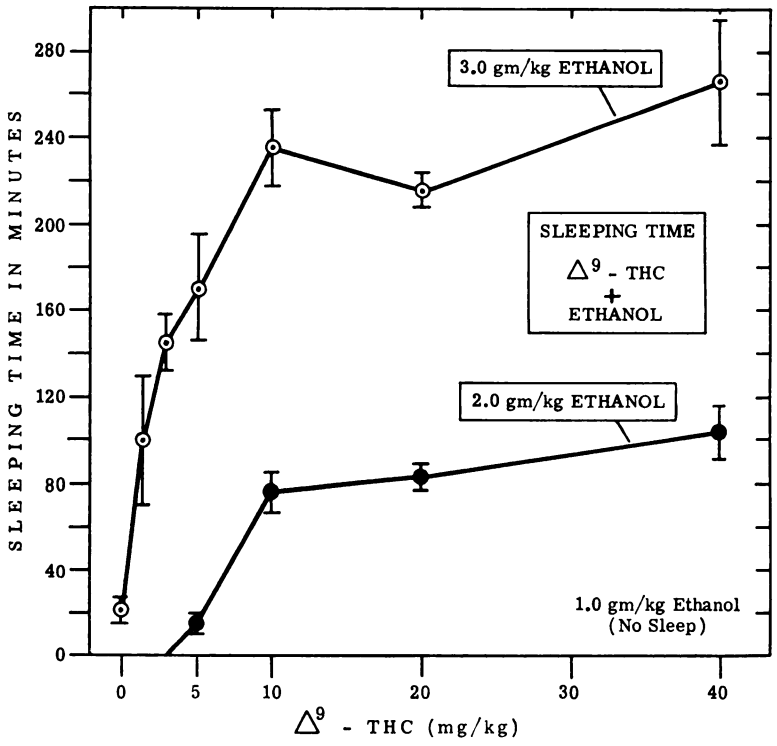

FIG. 2.

To investigate possible microsomal enzyme induction, Phillips *et al.* (unpublished observations) have administered Δ^9-THC in 5% albumin intraperitoneally to groups of male albino Swiss mice in doses of 0, 10, or 100 mg/kg daily for 3 days. A fourth group was given 100 mg/kg of phenobarbital. On the 4th day, all groups were given 60 mg/kg of pentobarbital and the duration of sleep was observed. Sleeping times in the control and Δ^9-THC groups were the same, while as expected, marked reduction in the sleeping time in the phenobarbital pretreated group was produced. Thus, phenobarbital pretreatment did not appear to stimulate Δ^9-THC metabolism or to shorten its sleeping time enhancement of pentobarbital. On the other hand, Sofia and Barry (12) have shown that Δ^9-THC prolongs barbital sleeping time in mice pretreated with SKF 525-A, a non-specific inhibitor of liver microsomal enzymes which may block the hydroxylation of Δ^9-THC.

Garriott *et al.* (3) have studied enhancement of hexobarbital sleeping time by natural Δ^9-THC and enhancement of *dl*-amphetamine-induced hyperactivity in male Swiss mice that weighed 18 to 22 g. The sleeping time enhancement reported was comparable to that found later by Phillips *et al.* (9) in rats. Motor activity after an intraperitoneal injection of 25 mg/kg of Δ^9-THC in 1% Tween 20 saline was reduced, but after the 4 mg/kg of *dl*-amphetamine were administered activity was enhanced. When the animals were pretreated with the Δ^9-THC 3 days before the *dl*-amphetamine administration, enhancement of motor activity

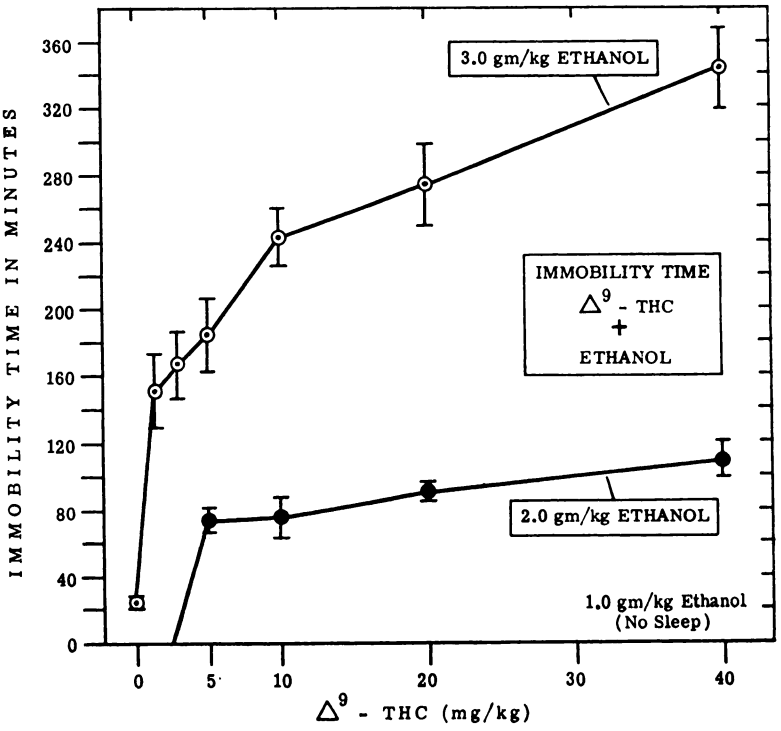

FIG. 3.

also occurred to a lesser degree but was greatly prolonged. An explanation of this apparent biphasic effect is not clear. The decrease in normal activity due to the THC alone occurred at the time of the greatest enhancement of *dl*-amphetamine activity by Δ^9-THC thereby suggesting a synergistic action.

Milzoff *et al.* (7) have studied the effect of Δ^9-THC on heart rate, respiratory rate and body temperature in the urethane anesthetized rat after doses of 0.625, 1.25, 2.5, 5.0 and 10 mg/kg. They have reported decreases in all the parameters measured. In a subsequent study (8), the experiment was repeated on rats given atropine. In this case, the decreases in heart rate were blocked and the decreases in blood pressure were reduced. Respiratory depression was unchanged.

Manno *et al.* (5) infused 0.1 to 4.0 mμ of Δ^9-THC per 3-min period into a perfused, isolated rat heart. As the dose was increased, the perfusion pressure also increased (vasoconstriction) but the force of contraction decreased. There was a return to control levels except with the highest dose.

Masur *et al.* (6) gave 2.5 mg/kg of Δ^9-THC suspended in saline and Tween 80 to both male and female rats 70 days old after 2 days of control exposure in an open field, 3 min per day. Defecation, grooming and rearing and ambulation were recorded. From the 4th to the 22nd day, the animals received daily intraperitoneal injections of 2.5 mg/kg of Δ^9-THC. The initial dose of the drug produced effects seen with depressant or tranquilizing agents, *i.e.*, amitryptyline, chlorpromazine

or barbiturates. After chronic administration, the parameters of rearing and grooming returned to control levels suggesting tolerance. However, the authors point out that enhancement of emotional reactivity occurred or, alternatively, that the initial depression masked the emotional reactivity produced by Δ^9-THC and, with the development of tolerance to this depression, emotional activity became evident. The possible biphasic action of Δ^9-THC is thus suggested although not unequivocally demonstrated.

Data collecting for the toxicology of Δ^9-THC in animals is accelerating and the reproducibility improving. Much more is required and is likely being generated, especially with chronic studies. Mechanism of action elucidation is also being pursued. Both phases are complicated by the diversity of activity already demonstrated for Δ^9-THC. Hopefully, the pharmacology and toxicology of marihuana will be better understood before too many more people are committed to its habitual use.

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