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Analgesic Activity of Δ^9-Tetrahydrocannabinol in the Rat and Mouse*

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Abstract. An analysis of the analgesic activity of Δ^9-tetrahydrocannabinol (Δ^9-THC) was carried out in rats and mice using both the hot plate and tail flick tests. In the rat, the dose-effect curve of Δ^9-THC was comparable to that of morphine in both tests. In the mouse, however, the THC dose-effect curves were more variable and less steep than the morphine dose-effect curves. THC was less potent than morphine in both tests in mice. THC analgesia reached its peak at one hour and had a longer duration of action than morphine.

Key words: Tetrahydrocannabinol — Morphine — Analgesia — Cannabis.

Introduction

The analgesic activity of marihuana has been a subject or recent debate. An early report indicated that cannabis had analgesic activity in rats only when near lethal doses were used (Davies *et al.*, 1946). Since then, Δ^9-tetrahydrocannabinol (Δ^9-THC) has been isolated from cannabis and subsequently synthesized (Mechoulam and Gaoni, 1965). Bicher and Mechoulam (1968) found Δ^9-THC to be an effective analgesic in mice and rabbits. Dewey *et al.* (1969) however, were unable to demonstrate analgesia with Δ^8- or Δ^9-THC in mice or rats. In the monkey, even though Δ^9-THC produced an analgesic effect the authors doubted whether the observed effect was truly analgesia (Scheckel *et al.*, 1968).

The present experiments in rats and mice were designed to compare the analgesic activity of Δ^9-THC with that of morphine over a wide range of doses in the hot plate and tail flick tests.

Methods

Male Sprague-Dawley rats (200–220 g) and male Dublin DBL/ICR mice (20–22 g) were used in these experiments. Analgesia was assessed

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by the hot plate (Eddy and Leimbach, 1953) and tail flick tests (D'Amour and Smith, 1941). A slide warmer (Fisher) maintained at 60° C was used for the hot plate. An inverted transparent polycarbonate cage ($6 \times 19 \times 10.5$ inches) was used to keep the animals on the hot plate. In the tail flick test, a Sylvania DCH projector lamp with a focal length of 5.7 cm was used to focus a beam of light on the unblackened tail. Light intensity was controlled by a variac (set to approximately 40 volts) to give control times of about 3—4 sec in both rats and mice. Reaction times were obtained for each animal in both the hot plate and tail flick tests 30 min before drug administration and at 30 min intervals after drug administration.

Pure synthetic Δ^9-THC was supplied as an ethanol solution sealed under nitrogen gas in brown glass ampules[1]. The ampules were stored at −4° C. The alcohol was evaporated under nitrogen and the gummy THC residue was suspended in a 2% Tween 80-saline solution by vigorous mixing and by subjecting the solution to high frequency vibration. This procedure required approximately one hour. The final suspension was either clear or slightly cloudy depending on the strength of the solution. Morphine sulfate was dissolved in saline. All doses are calculated as the free base.

All rats received the drugs intraperitoneally in a volume of 2 ml/kg. All mice received the drugs subcutaneously in a volume of 10 ml/kg.

Results

Analgesia in the Rat. The effect of Δ^9-THC on reaction time in the tail flick test is compared with that of morphine in Fig. 1. The peak effect obtained in each animal after drug treatment was compared with the control value and expressed as percent of control. Control reaction times averaged 3.2 ± 0.12 sec. A maximum reaction time of 15 sec was used. The peak effect of morphine usually occurred 30 min after drug administration and declined rapidly, whereas the peak effects of THC usually occurred 60 min after drug administration and declined slowly.

Although different dose levels of Δ^9-THC and morphine were used for comparison, it is obvious that the Δ^9-THC and morphine dose effect curves are superimposable. Following the 20 mg/kg dose of THC and the 16 mg/kg dose of morphine essentially all animals reached the maximum reaction time. Saline and Tween 80-saline control injections did not alter reaction times.

A similar comparison of the reaction times obtained in the hot plate test after Δ^9-THC and morphine treatment is shown in Fig. 2. The temperature of the hot plate was adjusted to give control reaction times averaging between 4—5 sec. A maximum reaction time of 20 sec was

1 Δ^9-THC was kindly made available by Dr. Daniel H. Efron, Psychopharmacology Research Branch, NIMH.

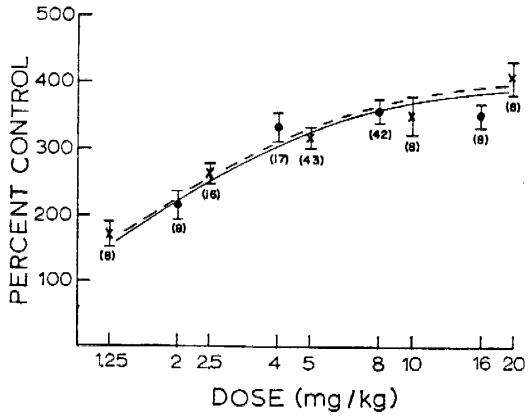

Fig. 1. Comparison of morphine and THC analgesic activity in the rat tail flick test. •—•, morphine; ×—×, Δ^9-THC. Animals were tested 30 min before injection to obtain control reaction times and at 30 min intervals after drug injection. Values shown are the means ± S.E. of the percent control values for each animal. Percent control was obtained by comparing the maximum reaction time observed in each animal after drug administration to its own control reaction time. Figures in parentheses represent the number of animals

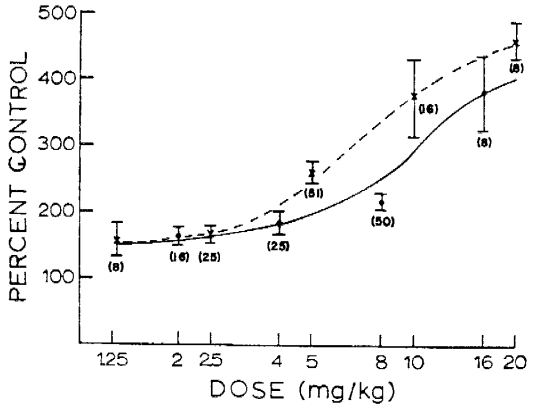

Fig. 2. Comparison of morphine and THC analgesic activity in the rat hot plate test. •—•, morphine; ×—×, Δ^9-THC. See Fig. 1 for further description

used. As in the tail flick test, the slope of the Δ^9-THC curve was comparable to that of morphine although the Δ^9-THC curve was shifted to the left. Thus in the hot plate test Δ^9-THC is slightly more potent than morphine.

An observation of interest was that some Δ^9-THC treated rats showed prolonged reaction times in both the hot plate and the tail flick test

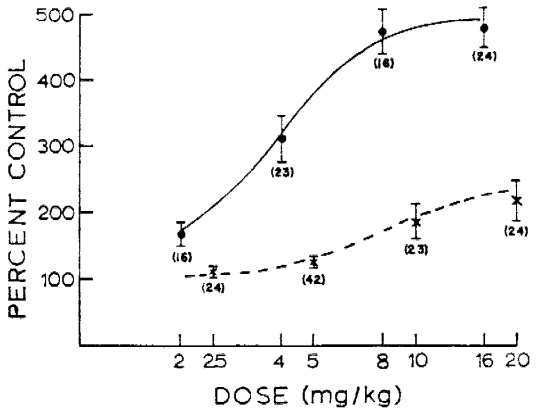

Fig. 3. Comparison of morphine and THC analgesic activity in the mouse tail flick test. •—•, morphine; × – ×, Δ^9-THC. See Fig. 1 for further description

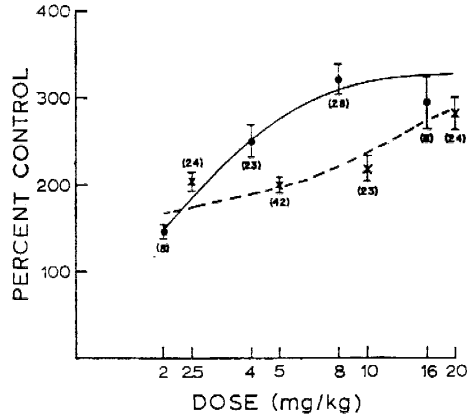

Fig. 4. Comparison of morphine and THC analgesic activity in the mouse hot plate test. •—•, morphine; × – ×, Δ^9-THC. See Fig. 1 for further description

although they began squealing and showing changes in respiration some seconds before actually making an appropriate response. This type of response was not seen with morphine. Thus, although Δ^9-THC is equipotent with morphine in both tests, there appears to be some difference between the behavioral responses of Δ^9-THC or morphine treated rats to the painful stimulus.

Analgesia in the Mouse. The tail flick test in the mouse was employed under conditions identical to those described for the rat. Control reaction time in the mouse was 3.1 $\pm$ 0.15 sec. The effect of Δ^9-THC and morphine

on reaction time in the mouse is shown in Fig. 3. Saline and Tween 80-saline injections had no significant effect on reaction times. As in the rat, the analgesic effect of Δ^9-THC usually reached its peak at 60 min after injection and declined slowly. The analgesic effect of morphine reached its peak 30 min after injection and declined rapidly. After subcutaneous injection in the mouse Δ^9-THC produced a flat dose-effect curve with a slope that differed from that of morphine. From these curves, Δ^9-THC can be seen to be considerably less potent than morphine.

In the mouse hot plate test, control reaction time was 5.4 ± 0.32 sec. The dose-effect curve obtained with Δ^9-THC was variable and very flat in comparison to the dose-effect curve for morphine (Fig. 4). In the mouse hot plate test, Δ^9-THC was again less effective than morphine.

Discussion

Cannabis has been reported to have analgesic activity in humans (Walton, 1938). Reports on the analgesic activity of cannabis or tetrahydrocannabinol in animals, however, have varied considerably. Davies *et al.* (1946) obtained a very flat dose-effect curve for analgesia in the rat tail flick test after intravenous injection of an extract of hashish. Pronounced analgesia was obtained only with doses close to the median lethal dose. Dewey *et al.* (1969) found that Δ^8- or Δ^9-THC were relatively inactive in the tail flick test in both the mouse and rat after oral or intravenous administration. Bicher and Mechoulam (1968), however, found Δ^9-THC to be about one-half as potent as morphine in both the hot plate and tail flick tests when administered intraperitoneally to mice. Our results with subcutaneous injection of Δ^9-THC in the mouse are comparable to those obtained by Bicher and Mechoulam in the mouse after intraperitoneal injection. Moreover, in the rat we have found Δ^9-THC to be equipotent to morphine in both the hot plate and tail flick test after intraperitoneal injection.

The differences in the reports on the analgesic activity of Δ^9-THC might be accounted for in part by differences in the routes of injection employed or in the strains of animals used. Grunfeld and Edery (1969) found that Δ^9-THC was effective in several behavioral tests in both rats and mice when it was injected intraperitoneally but not when it was injected subcutaneously. Although we obtained analgesia in the mouse after subcutaneous injection, the effect was not as great as that observed after intraperitoneal injection in the rat. Further studies comparing the effectiveness of Δ^9-THC after different routes of administration are essential.

Differences in the strains of animals used may also account for part of the variability in results reported by different laboratories. After obtain-

ing negative results in their analgesic studies in mice Dewey *et al.* (1969) obtained mice from the same supplier as the present authors and were able to obtain analgesic effects with Δ^9-THC (personal communication).

Another point of concern is the specificity and the validity of the analgesic test employed. Scheckel *et al.* (1968) noted analgesia in the squirrel monkey but expressed doubt that the effect observed was purely an analgesic effect. We observed squealing and changes in respiratory rate and depth in the Δ^9-THC treated rats during both the hot plate and tail flick tests although they did not respond appropriately. In rats, cannabis extracts and Δ^9-THC have been reported to produce vocalization in response to touch (Henriksson and Jarbe, 1971). The analgesic response cannot be explained by an impairment of locomotor activity, since although spontaneous locomotor activity appeared to be decreased in the Δ^9-THC treated animals (particularly the rats) all of the animals demonstrated some spontaneous and evoked locomotor activity. Thus, although Δ^9-THC is effective in some of the same analgesic tests as morphine is, further experiments will be required to determine whether it is an effective analgesic in a variety of tests and whether its mechanism of action is similar to that of morphine.

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