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Effect of Some Cannabinoids on Naloxone-Precipitated Abstinence in Morphine-Dependent Mice

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Abstract. Mice were rendered morphine-dependent by the subcutaneous implantation of a pellet containing 75 mg of morphine base; 72 h after the implantation, the animals were injected intraperitoneally either with vehicle or with various doses of Δ^9-tetrahydrocannabinol, Δ^8-tetrahydrocannabinol, cannabidiol, cannabinol, or 11-hydroxy-Δ^8-tetrahydrocannabinol. Thirty minutes after injection of the cannabinoids, the antagonist, naloxone HCl, was administered to induce the stereotyped withdrawal jumping syndrome. The dose of naloxone needed to induce withdrawal jumping in 50% of the animals (ED_{50}) was determined for each dose of the cannabinoids. All of the cannabinoids inhibited the naloxone-precipitated morphine abstinence as evidenced by an increase in the naloxone ED_{50}. Two additional signs of morphine abstinence, defecation and rearing behavior, were also suppressed by the cannabinoids. The relative effectiveness of the cannabinoids in inhibiting morphine abstinence appeared to be in the following order: Δ^9-tetrahydrocannabinol > Δ^8-tetrahydrocannabinol > 11-hydroxy-Δ^8-tetrahydrocannabinol > cannabidiol > cannabinol.

These data suggest that cannabinoids may be useful in facilitating narcotic detoxification.

Key words: Morphine-dependence – Naloxone – Δ^9-Tetrahydrocannabinol – Δ^8-Tetrahydrocannabinol – Cannabidiol – Cannabinol – 11-Hydroxy-Δ^8-tetrahydrocannabinol.

Current chemotherapy of narcotic dependence includes the use of either opiate antagonists to reinforce abstinence (Fink et al., 1971) or opiates and their synthetic derivatives, like methadone, in order to suppress most components of a narcotic abstinence syndrome (Collier et al., 1972). Naloxone, a narcotic antagonist, is used to block the euphoric effects of opiates and to reduce dependence by a process of negative reinforcement. However, it has relatively short duration of action and is not orally effective (Kurland and McCabe, 1976). In methadone-treatment program, the dosage of methadone is gradually increased to high daily doses, satisfying the subject's narcotic craving and, because of cross tolerance, blocking most of the euphoric effects of morphine and heroin (Dole et al., 1966; Goldstein, 1972). The methadone maintenance method is controversial because the long term effects are inconclusive and the person remains addicted.

Recently, Hine et al. (1975) have shown that Δ^9-tetrahydrocannabinol (Δ^9-THC) suppressed some of the naloxone-precipitated abstinence signs in morphine-dependent rats. The naloxone-precipitated withdrawal jumping, a highly characteristic sign of withdrawal in morphine-dependent mice (Way et al., 1969), is also inhibited by very low doses of Δ^9-THC (Bhargava, 1976). It was of interest to extend our previous findings to other cannabinoids, particularly to those that are devoid of psychotomimetic effects, e.g., cannabidiol and cannabinol. The present investigation reports the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabinol, cannabidiol, and 11-hydroxy-Δ^8-tetrahydrocannabinol, a metabolite of Δ^8-THC, on naloxone-precipitated withdrawal in morphine-dependent mice.

MATERIALS AND METHODS

Male Swiss-Webster mice, weighing 25–30 g (Scientific Small Animals, Arlington Heights, Illinois), were housed eight or nine per cage, with food and water ad libitum, and in temperature (23.3 ± 1.0° C), humidity (65 ± 2%), and light-controlled rooms (lighted 06.00–18.00). The animals were rendered tolerant to, and dependent on, morphine by the subcutaneous (s.c.) implantation of a morphine pellet containing 75 mg of morphine base according to the method of Way et al. (1969). At 72 h after the implantations,

either the control vehicle (1% Tween 80 in isotonic saline) or various doses of cannabinoids (as a suspension in the vehicle) were administered intraperitoneally (i.p.). To precipitate withdrawal, a dose of the antagonist, naloxone HCl, was injected s.c. 30 min after the vehicle or the cannabinoid administration. The morphine pellets were not removed from the mice. An inverse relationship exists between the degree of morphine-induced dependence and the dose of naloxone needed to evoke the withdrawal jumping response (Way et al., 1969). Immediately after naloxone administration, the mice were placed on a circular platform (56 cm high and 33 cm in diameter) and the number of mice jumping off the platform within a 15-min observation period was noted. Seven to ten animals were used for each of three doses of naloxone. The dose-response curve was drawn by linear regression analysis and the dose of naloxone required to precipitate jumping in 50% of the mice (ED_{50}) was determined. The naloxone ED_{50}, its 95% confidence limits, and the statistical test to establish significance were determined by the method of Litchfield and Wilcoxon (1949) using a Wang 700 computer.

Morphine abstinence was measured, comparing the effects of 2 doses, 5 and 10 mg/kg, of all the cannabinoids. The effect was expressed as per cent increase in naloxone ED_{50} in the experimental group (E) over that in the vehicle control group (C) by using the formula shown in Figure 2.

Qualitative observations were made on the defecation and rearing behavior exhibited during withdrawal by the experimental groups. The control group of morphine-dependent mice given naloxone showed 30 or more fecal boluses and 20 or more rearings within a 15-min observation period. The defecation and rearing in the experimental group was considered suppressed if the group showed ten or less fecal boluses and four or less rearings.

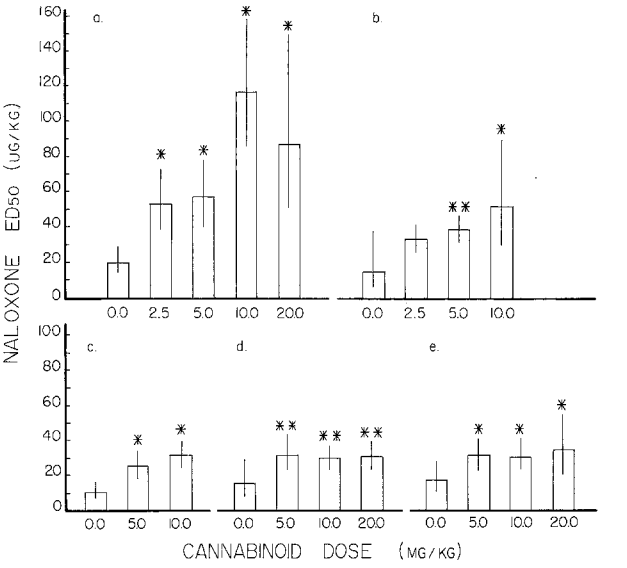

Fig. 1a–e. Effect of cannabinoids on naloxone ED_{50} in mice rendered dependent on morphine by pellet implantation. The vertical lines within the bar represent the 95% confidence limits of the ED_{50}. $^{*}P < 0.05$ vs. vehicle control; $^{**}P = 0.05$ vs. vehicle control. (a) Δ^9-tetrahydrocannabinol; (b) Δ^8-tetrahydrocannabinol; (c) 11-hydroxy-Δ^8-tetrahydrocannabinol; (d) cannabidiol; (e) cannabinol

RESULTS

Δ^9-Tetrahydrocannabinol at all doses tested (2.5, 5.0, 10.0, and 20.0 mg/kg) significantly ($P < 0.05$) inhibited the naloxone-precipitated withdrawal jumping in morphine-dependent mice as evidenced by an increase in the naloxone ED_{50} (Fig. 1a). A dose of 2.5 mg/kg of Δ^9-THC caused a 3-fold increase in the naloxone ED_{50} which was significantly different from that of the vehicle group. The naloxone ED_{50} in groups treated with 2.5 and 5.0 mg/kg of Δ^9-THC did not differ significantly. Administration of Δ^9-THC in a dose of 10 mg/kg caused a 6-fold increase in the naloxone ED_{50}. This response was significantly different from that of the vehicle control and also from that of the lower two dose groups. A dose of 20 mg/kg produced no greater response than a dose of 10 mg/kg; however, some signs of toxicity such as writhing and uncoordinated limb movements were noted with the 20 mg/kg dose. Δ^9-THC did not by itself induce withdrawal in morphine-dependent animals or cause any observable change in their behavior.

When administered to morphine-dependent mice, Δ^8-THC also inhibited the naloxone-precipitated withdrawal jumping. As shown in Figure 1b, doses of 2.5 and 5.0 mg/kg of Δ^8-THC increased the naloxone ED_{50} 2-fold, but only the higher dose (5 mg/kg) resulted in significant inhibition of abstinence ($P = 0.05$) resulted in significant inhibition of abstinence ($P = 0.05$). Δ^8-THC, when administered at a dose of 10 mg/kg, produced a significant ($P < 0.05$), 4-fold, increase in the naloxone ED_{50}.

11-Hydroxy-Δ^8-THC, a metabolite of Δ^8-THC, produced a highly significant inhibition of morphine abstinence signs. As shown in Figure 1c 11-hydroxy-Δ^8-THC produced a 2.5-fold increase in the naloxone ED_{50} at a dose of 5.0 mg/kg. This increase was significantly ($P < 0.05$) different from that of the vehicle control. Similarly a 10.0 mg/kg dose produced a 3-fold increase in the naloxone ED_{50}.

Administration of cannabidiol at doses of 5.0, 10.0 and 20.0 mg/kg also inhibited the naloxone-precipitated withdrawal jumping in the morphine-dependent mice. As shown in Figure 1d, a 2-fold increase in the naloxane ED_{50} was noted with all the doses of cannabidiol tested. A statistically significant increase ($P = 0.05$) in naloxone ED_{50} was observed with all the doses of cannabidiol. Cannabinol, when administered to morphine-dependent mice, inhibited the naloxone-precipitated withdrawal jumping. All the doses of cannabinol used (5.0, 10.0 and 20.0 mg/kg) increased the naloxone ED_{50} 2-fold as compared to the vehicle controls ($P < 0.05$) (Figure 1e). A dose of 5.0 mg/kg showed as good a response as a 20.0 mg/kg dose.

Defecation and rearing behavior observed during naloxone-precipitated withdrawal were also inhibited by the cannabinoids used. Mice implanted with

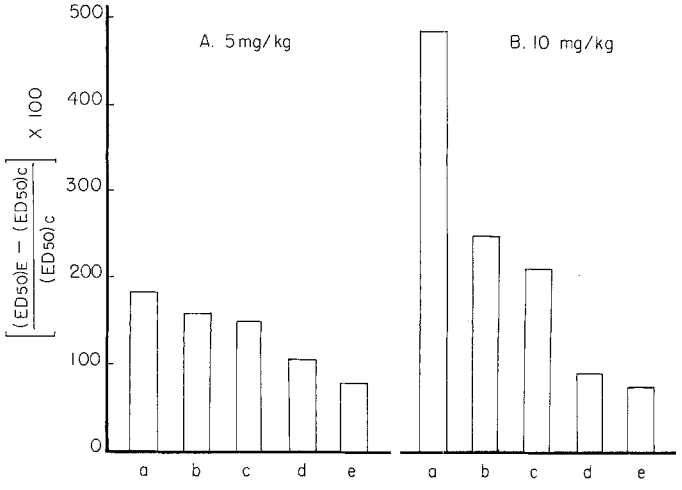

Fig. 2. Comparison of the inhibitory effects of two doses of cannabinoids on naloxone-precipitated withdrawal in morphine-dependent mice. The letters *a* through *e* represent the same cannabinoids as described in legend for Figure 1 and 'E' and 'C' represent the experimental and control groups

placebo pellets did not exhibit any morphine withdrawal sign following administration of naloxone, alone or in combination with any of the cannabinoids tested.

The relative effectiveness of the cannabinoids in inhibiting the morphine abstinence was compared by determining the effect of a fixed dose of all the cannabinoids on the increase in the naloxone ED_{50} over the respective control groups. As shown in Figure 2A, at a dose of 5.0 mg/kg of the cannabinoids, Δ^9-THC produced a 185% increase in the naloxone ED_{50} while cannabinol produced a 82% increase. Similarly, when a dose of 10 mg/kg of cannabinoids was used, Δ^9-THC increased the naloxone ED_{50} by 486% whereas cannabinol produced only a 76% increase (Fig. 2B). The other three cannabinoids exhibited intermediate activity. Thus, Δ^9-THC was the most active and cannabinol was the last active. The effectiveness of the cannabinoids in inhibiting morphine abstinence was in the same rank order for the two doses: Δ^9-THC > Δ^8-THC > 11-hydroxy-Δ^8-THC > cannabidiol > cannabinol.

DISCUSSION

The stereotyped withdrawal jumping, following treatment of morphine-dependent mice with naloxone, has been shown in several laboratories to be a highly characteristic sign of morphine dependence in mice (Way et al., 1969; Maruyama and Takemori, 1973) and rats (Francis and Schneider, 1971). The stereotyped jumping syndrome was inhibited by all of the cannabinoids studied, as was withdrawal defecation and rearing behavior.

The inhibition of morphine abstinence elicited by cannabinoids was evidenced by an increase in the dose of naloxone required to precipitate withdrawal jumping. In mice rendered dependent on morphine, cannabinoids generally caused a 2-fold to 6-fold increase in the naloxone ED_{50} depending upon the dose and the cannabinoid used. Δ^9-Tetrahydrocannabinol was found to be the most active cannabinoid in inhibiting the morphine abstinence syndrome whereas cannabinol was the least active. It is of interest that cannabidiol and cannabinol are devoid of psychotomimetic effects in humans (Hollister, 1974; Perez-Reyes et al., 1973; Carlini et al., 1973) and yet are active in suppressing the withdrawal signs in morphine-dependent mice as indicated by the present study.

It has been shown that Δ^9-THC can block some of the signs of naloxone-precipitated abstinence in rats rendered dependent by the implantation of one morphine pellet (Hine et al., 1975). The data indicated that THC in doses of 5.0 and 10.0 mg/kg significantly reduced the frequency of wet shakes and escapes. Similarly, defecation and diarrhea were also reduced. However, cannabidiol at a dose of 10.0 mg/kg was ineffective in reducing abstinence precipitated by naloxone. The reason for the different activity of cannabidiol in the mouse and in the rat in narcotic abstinence is not clear. From the study by Hine et al. (1975) it is evident that in the rat, of the nine withdrawal signs studied, three were suppressed by Δ^9-THC administration, although only a low degree of morphine tolerance and dependence develops after implantation of one or two morphine pellets in the rat (Bhargava et al., 1973).

The mechanisms by which the cannabinoids inhibit the naloxone-precipitated abstinence in morphine-dependent mice are not known at the present. The cannabinoids might be interacting with neurotransmitters in the central nervous system to produce this inhibition. The neurotransmitters [ACh, and DA] cyclic adenosine 3′,5′-monophosphate (cAMP) have been implicated in the expression of the abstinence syndrome. The expression of the naloxone-precipitated morphine abstinence has been shown to be associated with a decrease in whole-brain ACh levels in mice and rats (Bhargava and Way, 1975), an increase in whole-brain DA levels in mice and rats (Iwamoto et al., 1973), and an increase in whole-brain cAMP levels in rats (Collier and Francis, 1975).

Brain DA levels are reduced by Δ^9-THC (Graham et al., 1974). Δ^8-THC reduces striatal DA turnover (Littleton and MacLean, 1974). Askew et al. (1974) have found that Δ^9-THC and Δ^8-THC produced marked depletion of ACh in the rat brain whereas 11-hydroxy-Δ^8-THC had no effect on whole-brain ACh levels. Δ^8-THC was reported to have an effect

similar to that of anticholinergic agents in abolishing the behavioral inhibition of habituating experience in mice (Brown, 1971). Brown (1972) and Yoshimura et al. (1974), however, have shown that in some behaviors Δ^8-THC and Δ^9-THC may have anticholinesterase like activity. Changes in brain cAMP, following Δ^9-THC administration, show a biphasic effect. Low doses (0.1 to 1.0 mg/kg) cause elevation of cAMP levels, whereas doses in the range of 2 to 10 mg/kg cause depression of brain cAMP levels (Dolby and Kleinsmith, 1974). Apparently a relationship may exist between these biochemical effects and inhibition of naloxone-precipitated abstinence in morphine-dependent mice. The possibility that the cannabinoids may be substituting for morphine can not be excluded. Further studies are in progress to elucidate the mechanism of inhibition of morphine abstinence by the cannabinoids.

It is evident from the present investigation that in low doses, the cannabinoids can significantly inhibit some of the signs of the morphine abstinence syndrome, such as naloxone-precipitated withdrawal jumping, defecation, and rearing in morphine-dependent mice. Unlike methadone which is widely used in the treatment of narcotic addiction, none of the cannabinoids used has been shown to possess significant dependence liability. Therefore, the potential value of these cannabinoids or of some of their derivatives in narcotic detoxification warrants further exploration.

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