A Comparison of the Pharmacological Activity of Δ^9-Tetrahydrocannabinol and its Monohydroxylated Metabolites in Man

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the active principle of marihuana has been shown to be rapidly metabolized in vitro by the action of the liver microsomal enzymes to a number of primary mono-hydroxy intermediates. These include 11-OH-Δ^9-THC and 8β-OH-Δ^9-THC[1]. Recently, WALL et al.[2] have shown that in addition to the 11- and 8β-OH derivatives, 8α-OH-Δ^9-THC is found in man following the oral administration of Δ^9-THC. A limited number of studies have been carried out on the pharmacological activity of these metabolites. CHRISTENSEN et al.[3] found that 11-OH-Δ^9-THC was twice as potent as the parent compound in producing specific neurologic and behavioral responses when injected i.v. to mice. BEN ZVI et al.[4] have recently reported that the 8β- and the 8α-OH-Δ^9-THC have about $^1/_4$ the potency of Δ^9-THC when administered i.v. to rhesus monkeys. We have found the 8β-hydroxy to be approximately $^1/_3$ and the 8α-hydroxy to be $^1/_6$ as potent as Δ^9-THC when administered i.v. to mice in producing certain specific behavioral manifestations such as loss of locomotor coordination, general irritability, and changes in respiratory rate. This study reports a comparison of the effects of Δ^9-THC and its monohydroxylated metabolites when administered i.v. to man.

Forty-six normal, paid male volunteers ranging in age from 21 to 30 years were tested. 21 subjects were infused with Δ^9-THC, 11 subjects with 11-OH-Δ^9-THC, 9 subjects with 8β-OH-Δ^9-THC, and 5 subjects with 8α-OH-Δ^9-THC. All of these compounds were suspended in 25% human serum albumin by the technique reported elsewhere[5].

Subjects varied in their previous experience with marihuana from less than 1 cigarette/month to more than 5 cigarettes/week and were equally distributed in the groups. The subjects were hospitalized at the Clinical Research Unit of the North Carolina Memorial Hospital, Chapel Hill, N.C., and remained for 24 h until all the effects of the drugs had completely subsided. Respiration and heart rate were constantly recorded throughout the experiment by means of an Offner polygraph situated in a one-way screen room adjacent to the subjects' room. Blood pressure was determined at intervals throughout the experiment by the clinical auscultatory method.

Amount in μg/kg of Δ^9-THC or of its monohydroxylated metabolites injected i.v. to obtain certain specific effects

Drug		Perception of 'high'	Heart rate acceleration	Total dose
Δ^9-THC	Mean	18.77	23.29	53.14
$N = 21$	S.D.	7.51	8.09	16.16
11-OH-Δ^9-THC	Mean	16.60	17.19	46.99
$N = 11$	S.D.	4.17	8.56	22.09
8β-OH-Δ^9-THC	Mean	48.64	137.37	183.06
$N = 9$	S.D.	15.16	84.92	67.35
8α-OH-Δ^9-THC	Mean	>186.36	>186.36	186.36
$N = 5$	S.D.	—	—	11.47

Only 15 mg per subject of 8α-OH-Δ^9-THC was available. That is the reason why no more of this compound was infused.

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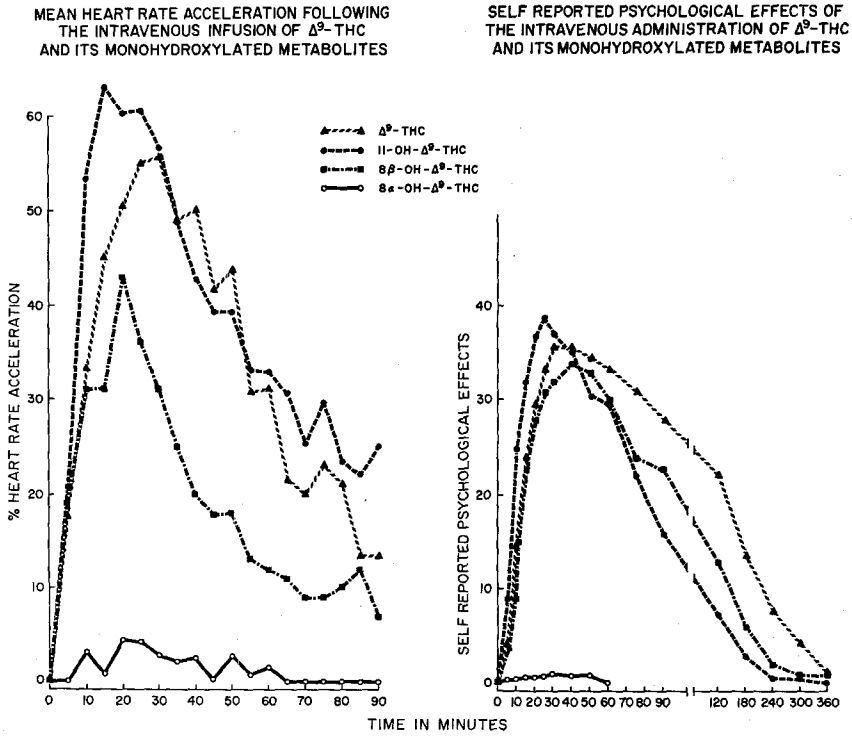

The values recorded in this figure were obtained by infusing 8β-OH-Δ^9-THC at twice the rate and 8α-OH-Δ^9-THC at 4 times the rate of Δ^9-THC and its 11-hydroxy derivative. The units of measurement of the subjective score are the number of squares of a standard graph paper by which the subjects elected to rate themselves. The curves represent the mean of all the subjects tested. Statistical analysis of the differences was based on the area under the curves, thus taking into consideration the intensity and duration of effects.

To obtain the subjective evaluation of drug effects, that is of marihuana-like 'high', whether pleasent or unpleasant, we asked the subjects to rate themselves in a graph form provided for them. This rating was obtained at appropriate intervals for 6 h following drug administration. No specific instructions were given for these ratings, and each subject was free to utilize whathever criterion he wished. We found that although there were variation in rating the magnitude of 'high', the pattern of the psychological experience in time was consistently similar.

Subjects were told that initially they would be i.v. infused with a drug-free solution (normal saline), and that at some unspecified time, it would be replaced with the preparation containing either Δ^9-THC or any of its monohydroxylated derivatives. The replacement of solutions without the subjects' awareness was possible because the Harvard constant infusion pump utilized for injection was located in the observation room. The subjects were instructed to report the moment they felt the action of the drug, that is the initial perception of marihuana-like effects, and to ask for the termination of the infusion as soon as they felt they had arrived at their desired level of 'high'. The volunteers were encouraged to receive the largest amount of the drug that they could comfortably tolerate. By giving the subjects control as to the amount of drug to be injected and by the constant recording of vital signs, we insured the safety and confidence of the volunteers. Likewise, progressive administration of the drugs mimics their actual pattern of use, since they are most frequently inhaled until the user decides that he has reached his desired level of 'high'. Variable times of placebo injection were used ranging from 15–25 min, and the subjective ratings were always base line indicating that there were no placebo responses under our experimental conditions. After the placebo injection, Δ^9-THC and 11-OH-Δ^9-THC were infused at the rate of 0.2 mg/min (0.92 ml/min) until the subject decided that he had achieved his desired level. Infusion of 8β-OH-Δ^9-THC at this rate in the first subject tested failed to produce any marihuana-like effects. For this reason, the rate of administration was increased to 0.46 mg/min (2.23 ml/min) for the remaining 9 subjects. Infusion of 8α-OH-Δ^9-THC at this higher rate of administration failed to produce any effects in the first subject tested, and the rate of administration was increased to 0.92 mg/min (4.46 ml/min) for the remaining 5 subjects.

The Table illustrates the doses necessary in µg/kg to perceive the action of the drugs, to accelerate the heart rate more than 25% over the initial level, and to achieve the desired level of 'high'. The results indicate that the Δ^9-THC and its 11-hydroxylated metabolite have similar potencies; the 8β-hydroxylated metabolite is less potent, and the 8α-hydroxylated derivative appears to have no potency at the dose and rate of infusion utilized.

The subjective experience of marihuana-like 'high' was rated as equal both in duration and intensity among the subjects receiving the Δ^9-THC and its 11- and 8β-hydroxylated metabolites (Figure). At the end of the experiment, subjects infused with either Δ^9-THC or the 11-OH-Δ^9-THC reported that the 'high' had been the most intense they had ever experienced, while those infused with the 8β-OH-Δ^9-THC did not. This diminished potency of the 8β metabolite is further illustrated by the fact that 5 of the 9 subjects infused with it never reached their desired or maximum level of 'high'.

The i.v. infusion of Δ^9-THC and its 11-hydroxylated metabolite produced a marked acceleration of the heart rate and although there was a difference in the magnitude (Figure), it was not statistically significant. The tachycardia produced by the 8β-OH-Δ^9THC was of less magnitude and duration ($p > 0.01$) even though it was infused at a faster rate. The 8α-OH-Δ^9THC did not accelerate the heart when infused at 4 times the rate of that of the Δ^9-THC. This failure to accelerate the heart parallels the absence of psychological effects and demonstrates the lack of pharmacological activity of the 8α-hydroxylated metabolite.

The finding that hydroxylation at the 11-position did not significantly increase the potency of Δ^9-THC casts reasonable doubt on the hypothesis that the 11-hydroxy derivative is the active form of the parent compound. Thus, it if were necessary for Δ^9-THC to be metabolized to the 11-hydroxy compound to exert its marihuana-like action, the injection of the 11-hydroxy derivative at the same rate should have produced more intense effects more quickly than those produced by the parent compound, but this did not occur. Furthermore, after the i.v. injection of Δ^9-THC, we have found that the levels of the 11-OH-Δ^9-THC were never more than 2% of the total cannabionoids present in the plasma (results will be published elsewhere). It is unlikely that this minute amount of the 11-hydroxy metabolite is responsible for the effects of Δ^9-THC.

Resumen. Se hizo un estudio comparativo de la capacidad del Δ^9-tetrahidro-cannabinol y de sus metabolitos monohidroxilados en producir effectos similares a los de la marihuana cuando son inyectados intravenosamente a humanos. Se encontro que la hidroxilacion en la posición 11 no cambio la potencia, en la posición 8β la redujo, y en la posición 8α la abolio por completo.

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The Activating Action of Acetylcholine and Pilocarpine on the Oxidation of Luminol

The chemiluminescent oxidation of luminol (3-aminophtalhydrazide) with aqueous alkaline hydrogen peroxide and an 'activating' agent[1] or 'co-oxidant'[2] is a very complex multistep reaction. A number of detailed mechanisms have been proposed for this reaction by various workers. In most of them there are two common points: 1. the first step of the reaction involves the oxidation of the anion of luminol by the activator and 2. the key