THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 200, No. 2

Printed in U.S.A.

9-*nor*-9β-HYDROXYHEXAHYDROCANNABINOL, A CANNABINOID WITH POTENT ANTINOCICEPTIVE ACTIVITY: COMPARISONS WITH MORPHINE [1, 2]

ALAN S. BLOOM,[3] WILLIAM L. DEWEY, LOUIS S. HARRIS AND KEITH K. BROSIUS

Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

Accepted for publication September 9, 1976

ABSTRACT

BLOOM, ALAN S., WILLIAM L. DEWEY, LOUIS S. HARRIS and KEITH K. BROSIUS: 9-*nor*-9β-Hydroxyhexahydrocannabinol, a cannabinoid with potent antinociceptive activity: Comparisons with morphine. J. Pharmacol. Exp. Ther. **200:** 263–270, 1977.

The effects of (±)9-*nor*-9β-hydroxyhexahydrocannabinol (β-HHC) on tail-flick test activity and the accumulation of newly synthesized dopamine and norepinephrine were studied in the male albino mouse. The same parameters were also studied in naloxone-pretreated and morphine-tolerant mice. β-HHC was about equipotent with morphine in the mouse tail-flick (ED50 = 7.12 mg/kg). The cannabinoid also produced dose-dependent increases in the accumulation of newly synthesized DA and NE. Pretreatment with 2 mg/kg of naloxone antagonized both the tail-flick activity and blocked the increases in catecholamine synthesis produced by β-HHC. Cross-tolerance between β-HHC and morphine did not exist in regard to either tail-flick activity or increased catecholamine synthesis. These studies suggest that β-HHC may share some properties with the narcotic analgesics but that significant differences exist. Furthermore, these studies offer further evidence for the involvement of catecholamine containing neurons in the central mediation of the tail-flick response.

There is a disagreement in the literature regarding the analgesic properties of Δ^9-tetrahydrocannabinol (Δ^9-THC) and other cannabinoids. In the rat, Δ^9-THC is active in both the hot-plate and tail-flick tests (Buxbaum, 1972; Gallager *et al.*, 1972). However, Buxbaum (1972) reported that there appeared to be a qualitative difference between the behavioral responses of Δ^9-THC- or morphine-treated rats to painful stimuli. Δ^9-THC and some other cannabinoids have been reported to have activity in both the mouse hot-plate and mouse abdominal constriction tests, but in general appear to be less potent than morphine (Bicher and Mechoulam, 1968; Dewey *et al.*, 1970a, 1972; Buxbaum, 1972; Chesher *et al.*, 1973; Sofia *et al.*, 1973; Wilson and May, 1975). However, it appears that Δ^9-THC is significantly less active than morphine in the mouse tail-flick test (Buxbaum, 1972; Dewey *et al.*, 1970a, 1972).

Received for publication February 23, 1976.
This work was supported in part by U.S. Public Health Grants DA-00490 and T32 DA-05008.
[2] Presented in part at the Meetings of the Society for Neuroscience, New York, N. Y. November 1975.
[3] Supported by U.S. Public Health Service Grant T32 DA-05008. Present address: Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisc. 53201.

Send reprint requests to: William L. Dewey, Box 26, MCV Station, Medical College of Virginia, Richmond, Va. 23298.

These discrepancies between the results reported on the effect of Δ^9-THC in the tail-flick test have been attributed to strain differences of animals, methods of testing and other methodological differences. Our contention that Δ^9-THC is significantly less potent than morphine is supported by the observation that (±)-9-*nor*-9β-hydroxyhexahydrocannabinol (β-HHC) a synthetic analog of Δ^9-THC (fig. 1) has been found to be about equal to morphine in antinociceptive potency in the mouse tail-flick test. The α-isomer (α-HHC) of this compound (fig. 1) is inactive in the mouse tail-flick test (Martin *et al.*, 1976).

Narcotic analgesics increase the synthesis of brain catecholamines in the rat and mouse (Clouet and Ratner, 1970; Smith *et al.*, 1972). We have shown previously that a correlation exists between the antinociceptive activity as measured in the mouse tail-flick test and increased brain catecholamine synthesis produced by narcotic analgesics (Bloom *et al.*, 1976a). The present experiments were performed to further test the hypothesis that catecholamine-containing neurons in the brain are involved in the central mediation of the mouse tail-flick response and possibly antinociception in other species. The good correlation between activity of drugs in the tail-flick test and antinociception in other species including man support the possible generalization of this hypothesis (Archer *et al.*, 1964).

In the present study, the effects of α- and β-HHC in the mouse tail-flick test were compared with their effects on endogenous levels and synthesis rates of brain dopamine and norepinephrine. In addition, the effects of β-HHC on these systems were also examined in naloxone-pretreated and morphine-tolerant mice in order to determine whether similarities exist between the mechanisms of action of β-HHC and the narcotic analgesic. The possibility of similarities between behaviorally active cannabinoids and morphine has been suggested in the report of Hine *et al.* (1975).

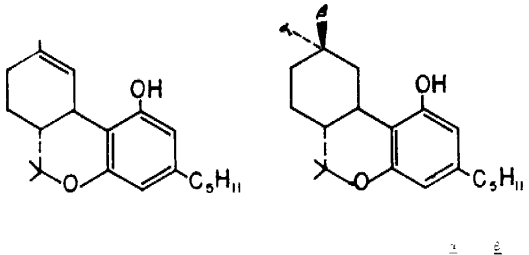

FIG. 1. Structure of Δ^9-tetrahydrocannabinol and the 9-*nor*-9-hydroxyhexahydrocannabinols.

Methods

Male random bred albino mice (ICR strain, Dub
lin Farms, 20–30 g) were used in all studies. The
were housed six per cage and kept in a temperatur
controlled room with the lights on from 7:00 A.M. t
7:00 P.M. daily. All animal experiments were per
formed between 1:00 P.M. and 4:00 P.M. to contro
for diurnal variations in pain sensitivity and cate
cholamine synthesis.

All injections were given subcutaneously in a vo
ume of 0.1 ml/kg b.wt. β-HHC, α-HHC and Δ^9-THC
were administered using a emulphor-ethanol-salin
vehicle (Cradock *et al.*, 1973). Morphine sulfate an
naloxone were dissolved in saline. Morphine toler
ance was produced by the subcutaneous implanta
tion of a pellet containing 75 mg of morphine alka
loid as described by Way *et al.* (1969).

The antinociceptive effect of the cannabinoids an
morphine was assessed using the tail-flick method
D'Amour and Smith (1941) as modified by Dewey e
al. (1970b). Antinociceptive potencies were calcu
lated as the percentage of maximum possible effec
(% MPE) (Harris and Pierson, 1964) using a 10
second cut-off time and a control reaction time of 2 t
4 seconds. ED50 values and 95% confidence limit
were determined by the method of Litchfield an
Wilcoxon (1949).

In the biochemical studies mice were sacrificed b
rapid decapitation 30 minutes after the subcuta
neous injection of 50 μCi of ^{3}H-tyrosine (30–50 C
mmol, New England Nuclear Corporation, Bostor
Mass.). The brains were removed, weighed and ho
mogenized in 3 ml of cold 0.4 N perchloric acid afte
the cerebellum was dissected away. The homoge
nate was centrifuged at 10,000 × *g* for 10 minute
and the supernatant was decanted and saved. Th
pellet was rehomogenized in an additional 3 ml
perchoric acid and centrifuged. The supernatant
were then combined and frozen until assayed.

Chemical methods. Endogenous brain tyrosin
was measured in a 0.5-ml aliquot of the brain tissu
homogenate supernatant by the fluormetric metho
of Waalkes and Udenfriend (1957). ^{3}H-tyrosine wa
determined by separation from another 0.5-ml al
quot of the supernatant on Dowex 50 W (H^+) io
exchange columns using a slight modification of th
technique of Weiner and Rabadjija (1968) and coun
ing a portion of the column eluate by liquid scintilla
tion spectrophotometry.

Catecholamines were separated from another
ml of the brain homogenate supernatant by absorp
tion onto Woelm alumina at pH 8.4 to 8.6 and el

tion from it with 5 ml of 0.2 N acetic acid after three successive distilled water washes. Endogenous levels of dopamine and norepinephrine were measured in a 1-ml aliquot of the alumina eluate by a slight modification of the fluorometric method of Shellenberger and Gordon (1971). ^{3}H-dopamine and ^{3}H-norepinephrine were separated from 3 ml of the alumina eluate using Dowex 50 W (Na^+) ion exchange columns. ^{3}H-norepinephrine and ^{3}H-dopamine were eluted from the columns with 1 and 2 N HCl, respectively, and measured by liquid scintillation spectrophotometry.

Dopamine (DA) and norepinephrine (NE) synthesis rates were estimated by the use of an accumulation index which was derived from the work of Zigmond and Wurtman (1970) and takes into account the specific activity of ^{3}H-tyrosine at the time of sacrifice. They reported that the accumulation of ^{3}H-catecholamines is dependent on the actual synthesis rates of the catecholamines, the specific activity of the tyrosine precursor and the utilization of the catecholamines, and that it approximates the synthesis of the catecholamines. Because this method requires only a single determination, does not change the steady state and can reflect rapid changes in rates of catecholamine synthesis (Sedvall *et al.*, 1968), it is an appropriate technique for assessing the effects of pharmacological agents on brain catecholamines.

The accumulation of newly synthesized NE and DA was calculated using the following equation:

$$\frac{\text{Accumulation of newly synthesized DA or NE} = \text{dpm } ^3\text{H DA or NE/g brain weight}}{\text{dpm } ^3\text{H-tyrosine}/\mu\text{g endogenous tyrosine}}$$

The values obtained from the equation are then multiplied by a constant that accounts for the difference in molecular weight between tyrosine and dopamine or norepinephrine and the loss of one tritium atom in the conversion of 3,5-^{3}H-tyrosine to catecholamine. The accumulation of newly synthesized dopamine or norepinephrine is then expressed in terms of micrograms of the amine per gram of wet brain weight. All biochemical data were analyzed by analysis of variance and Dunnett's modification of the *t* test (Winer, 1972).

Results

Activity of β-HHC and morphine SO_4 in the mouse tail-flick test. β-HHC was equipotent to morphine in the mouse tail-flick test 30 minutes after their subcuteneous administration (fig. 2). The slope functions of the two dose-response curves also did not differ. The ED50 for β-HHC was 7.12 mg/kg, and for morphine 5.28 mg/kg. α-HHC and Δ^9-THC were much less active than β-HHC in the mouse tail-flick test (table 1). Furthermore, even very high doses of Δ^9-THC (400 mg/kg) produced only a 62% effect, which was no greater than the effect produced by 150 mg/kg of the drug. The dose-response curve for Δ^9-THC asymptotes unlike those obtained for morphine and β-HHC.

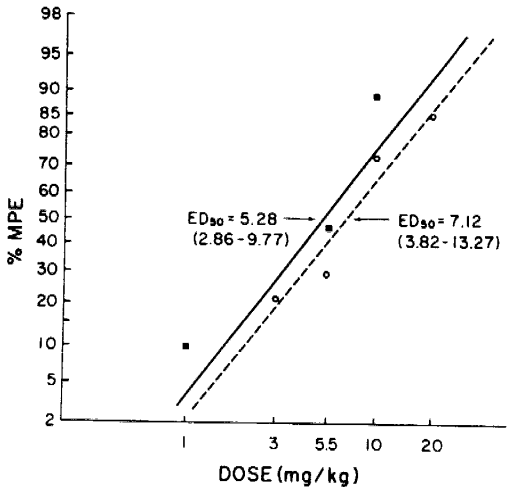

FIG. 2. Tail-flick activity of morphine (■—■) and β-HHC (O---O). Each point consists of the data from six mice. ED50 values are shown with their 95% confidence limits.

TABLE 1

Tail-flick activity of Δ^9-THC and α-HHC

Dose	Tail-Flick Activity Δ^9-THC	% MPE α-HHC
mg/kg		
20	16	9
30	5	
50		14
100	17	18
150	63	
200	58	25
400	62	

[a] Six animals were tested at each dose. Testing was performed 30 minutes after subcutaneous drug injection.

Effect of β-HHC, α-HHC, and Δ^9-THC on the accumulation of newly synthesized DA and NE. β-HHC produced dose related increases in the accumulation of newly synthesized dopamine ($f = 6.18$; $P < .01$) and norepinephrine ($f = 2.62$; $P < .05$) (fig. 3). Treatment with 20 mg/kg of β-HHC produced a 117% increase in the accumulation of newly synthesized dopamine ($P < .005$) and a 68%

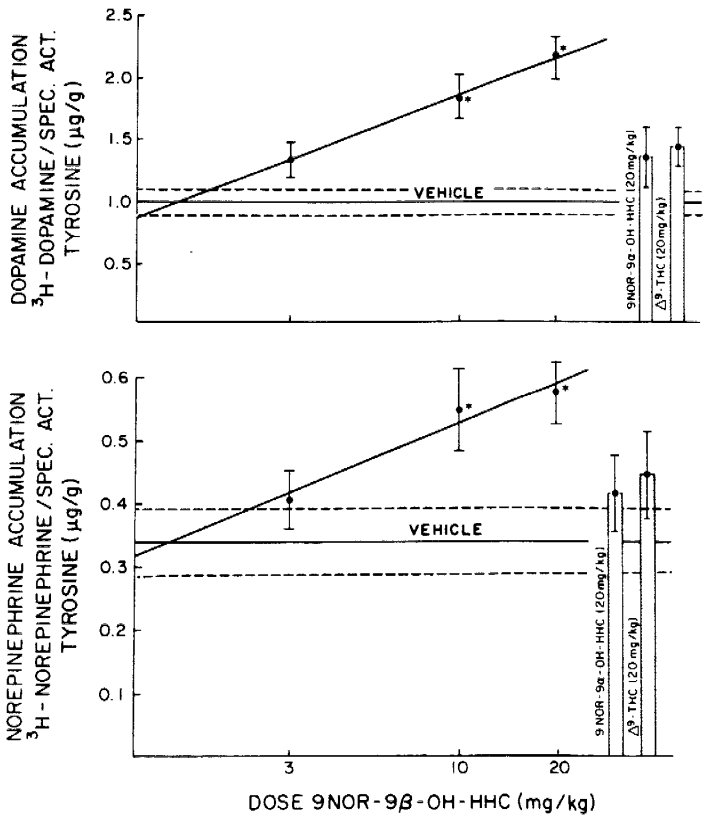

FIG. 3. Effects of β-HHC, α-HHC and Δ^9-THC on the accumulation of newly synthesized dopamine and norepinephrine. Drugs and ^{3}H-tyrosine were injected s.c. 30 minutes before sacrifice. All values shown are the mean ± the standard error of the mean. There were six animals in each group. The β-HHC dose-response lines were determined by linear regression analysis. *P < .05 when compared to vehicle-injected controls.

increase in the accumulation of newly synthesized norepinephrine (P < .05). Significant increases were also observed after treatment with 10 mg/kg of β-HHC. A 20 mg/kg dose of either α-HHC or Δ^9-THC produced only small increases in the accumulation of newly synthesized dopamine and norepinephrine that did not reach statistical significance.

Analysis of variance indicated that treatment with the cannabinoids used in this study did not produce significant changes in endogenous levels of dopamine or norepinephrine when compared to vehicle controls (1.378 ± 0.076 μg/g and 0.520 ± 0.018 μg/g, respectively). Similarly the drug treatments did not significantly alter the precursor pool. The endogenous brain tyrosine level in the vehicle control group was 17.09 ± 1.64 μg/g and the specific activity of ^{3}H-tyrosine in the brain was 10445 ± 1350 dpm/μg of endogenous tyrosine.

Effect of naloxone on the antinociception and increased accumulation of newly synthesized DA and NE produced by β-HHC. Mice were injected subcutaneously with 2 mg/kg of naloxone or saline 10 minutes before the injection of each four doses between 3 and 30 mg/kg of β-HHC. The animals were tested on the tail-flick apparatus or sacrificed 30 minutes after the second injection. Naloxone produced a significant shift (P < .05) in the dose-response curve for β-HHC in the tail-flick test (fig. 4). Naloxone also significantly (P < .05) antagonized the effects of β-HHC on the accumulation of newly synthesized dopamine and norepinephrine (fig. 4).

Effects of β-HHC in morphine pellet-implanted animals. Mice were implanted with either a 75-mg morphine pellet or an inert placebo pellet in an attempt to determine whether there was cross-tolerance between

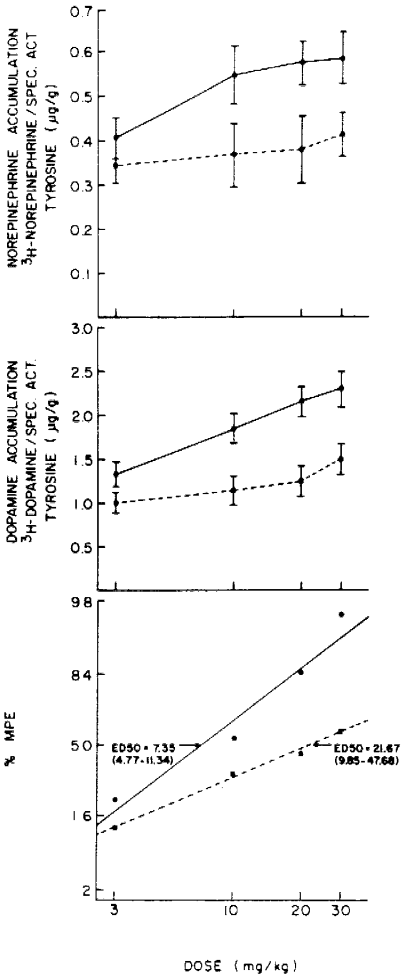

FIG. 4. Effects of naloxone (2 mg/kg) on the tail-flick activity and increased catecholamine synthesis produced by β-HHC. Mice were injected with naloxone 10 minutes before the injection of β-HHC. There were 12 mice in each group in the biochemical studies. Vehicle control values were 0.996 ± 0.102 μg/g of DA and 0.340 ± 0.052 μg/g of NE for the β-HHC only groups (●——●), and 0.936 ± 0.095 μg/g of DA and 0.312 ± 0.045 μg/g of NE for the naloxone plus β-HHC group (■---■). The values shown are the mean ± the standard error of the mean.

morphine and β-HHC in either tail-flick activity or increased accumulation of newly synthesized catecholamine. At 72 hours after pellet implantation, the ED50 for morphine in the tail-flick test was 5 times greater in mice implanted with morphine pellets than in placebo-implanted controls ($P < .05$) (table 2). However, the ED50 for β-HHC was less in morphine pellet-implanted animals than in those implanted with placebo pellets, although this difference was not statistically significant.

The effects of morphine in β-HHC on the accumulation of newly synthesized dopamine and norepinephrine in pellet-implanted mice are shown in table 3. The accumulation of newly synthesized dopamine was increased 88% 30 minutes after the injection of 20 mg/kg of morphine in placebo pellet-implanted mice when compared to vehicle injected placebo pellet controls. Similarly the accumulation of newly synthesized norepinephrine was increased 77%. The effects of the morphine injection on catecholamine synthesis in animals implanted with morphine pellets did not differ significantly from those injected with vehicle. β-HHC produced significant increases in the accumulation of newly synthesized dopamine (70%) and norepinephrine (66%) in placebo pellet-implanted mice. The effects of 20 mg/kg of β-HHC on catecholamine synthesis were slightly greater in the morphine pellet-implanted mice than in the placebo-implanted groups. As mentioned above, β-HHC also had more effect in increasing tail-flick latency in morphine pellet mice than in placebo pellet-implanted animals.

Discussion

The results of these studies confirm that (±)-9-*nor*-9β-hydroxyhexahydrocannabinol possesses morphine-like potency in the mouse tail-flick test. Like morphine (Smith *et al.*, 1972) and other narcotic analgesics (Bloom *et al.*, 1976a), β-HHC also increases the accumulation of newly synthesized dopamine and norepinephrine formed from ^{3}H-tyrosine. When the 9-hydroxyl group was changed from the β or equatorial position to the α or axial position, both the tail-flick activity and the increased accumulation of newly synthesized catecholamines were lost. Furthermore, stud-

TABLE 2

Tail-flick activity of morphine and β-HHC in placebo- and morphine pellet-implanted mice[a]

	ED50 and 95% Confidence Limits	
	Placebo pellet	Morphine pellet
	mg/kg	
Morphine	3.40 (1.20–9.64)[b]	17.28 (9.21–32.44)[b]
β-HHC	7.20 (1.98–20.62)	3.83 (1.18–12.61)

[a] Mice were implanted with pellets containing 75 mg of morphine or the inert binder 72 hours before testing. Six mice were used at each dose.

[b] Significantly different from each other ($P < .05$).

TABLE 3

Effects of β-HHC and morphine on the accumulation of newly synthesized dopamine and norepinephrine morphine- and placebo pellet-implanted mice[a]

Treatment	^{3}H-DA/Specific Activity Tyrosine	^{3}H-NE/Specific Activity Tyrosi
	μg/g ± SEM	*μg/g ± S.E.M.*
Saline	1.226 ± 0.166	0.319 ± 0.060
Placebo pellet and saline	1.316 ± 0.147	0.310 ± 0.037
Placebo pellet and morphine (20 mg/kg)	2.307 ± 0.209[b, c]	0.566 ± 0.047[b, c]
Morphine pellet and morphine (20 mg/kg)	1.425 ± 0.215[c]	0.382 ± 0.081[c]
Placebo pellet and HHC (20 mg/kg)	2.192 ± 0.562[b]	0.530 ± 0.062[b]
Morphine pellet and HHC (20 mg/kg)	2.282 ± 0.342[b]	0.618 ± 0.125[b]

[a] Six mice in each group.
[b] Significantly different from saline injected controls ($P < .05$).
[c] Significantly different from each other ($P < .05$).

ies performed in our laboratories with the small amount of the (−)-isomer of β-HHC that was available suggest that this isomer is responsible for the antinociceptive activity as it is about twice as active as the racemic mixture (Aceto *et al.*, 1975). These results have been confirmed by Wilson *et al.*, (1976).

The results of the present study are in agreement with our previous reports (Dewey *et al.*, 1970a, 1972) on the relative inactivity of Δ^9-THC in the mouse tail-flick test. We observed minimal antinociceptive activity (16% MPE) 30 minutes after the subcutaneous injection of 20 mg/kg of Δ^9-THC. Higher doses of Δ^9-THC (150–400 mg/kg) were more active in the tail-flick test (about 60% MPE for all doses), but there was a rather flat dose-response relationship. β-HHC was approximately 10 times as active as Δ^9-THC in the mouse tail-flick test and also produced a qualitatively different dose-response curve.

Gross observation of mice treated with β-HHC suggested that it produced a greater decrease in spontaneous motor activity than the same dose of either morphine or Δ^9-THC. β-HHC-treated mice also appeared to have increased rear leg extension activity. However, it is not likely that the tail-flick activity of β-HHC is merely the result of a decrease in the spinal reflex measured since the compound is as active as morphine in the *p*-phenylquinone abdominal constriction test (Aceto *et al.*, 1975). Furthermore it is unlikely that the tail-flick activity is due to a general increase in the sensory thresholds for all modalities since anesthetic doses of pentobarbital are inactive in this procedure (unpublished data). On t other hand, it is possible that the antinocice tice activity of β-HHC in the mouse tail-fl test may be due at least in part to supraspi effects of this drug as is probably the case w morphine (Dewey *et al.*, 1969).

β-HHC is significantly more active than THC in increasing the activity of catech amine-containing neurons as determined measuring the accumulation of newly synth sized dopamine and norepinephrine. In t present study 20 mg/kg of Δ^9-THC produc increases in the accumulation of newly s thesized dopamine and norepinephrine th were not statistically significant and we about equal to those observed after only 3 m kg of β-HHC. Δ^9-THC and other cannabis de vitives increase the accumulation of new synthesized catecholamines in rat (Maitre *al.*, 1970, 1972) and in mouse (Bloom *et a* 1976b). However, this increase was of a less magnitude than those produced by β-HHC the mouse. The greater effect of β-HHC tha Δ^9-THC on brain catecholamine-containi neurons may serve at least in part to expla the antinociceptive activity of this compou in the mouse tail-flick test if these neurons a involved in the central mediation of this r sponse as we have hypothesized (Bloom *et a* 1976a).

Naloxone antagonizes the activity of the na cotic analgesics (Blumberg *et al.*, 1966) a blocks their effects on catecholamine synthes (Smith *et al.*, 1972). In the present study, it w observed that naloxone significantly antag nized the tail-flick activity of β-HHC, and si

nificantly blocked the increased accumulation of newly synthesized dopamine and norepinephrine produced by β-HHC. However, it appears that there is a decrease in the naloxone effect at the highest dose of β-HHC tested. Naloxone also antagonizes the analgesic activity of 11-OH-Δ^8-THC in the hot-plate test (Wilson and May, 1975). These data suggest that the narcotic analgesics and certain cannabinoid compounds may have some common sites of mechanism of action which are blocked by naloxone. These data also support the hypothesis that catecholamines are involved in the central mediation of the tail-flick response.

Smith *et al.* (1972) reported that tolerance could be produced to the effects of morphine on brain catecholamines and that cross-tolerance was present to levorphanol. There does not appear to be cross-tolerance between morphine and β-HHC. Mice implanted with morphine pellets were rendered tolerant to both the antinociception and increased catecholamine synthesis produced by morphine. The potency of β-HHC was not decreased in morphine pellet-implanted animals and may have been slightly increased. This finding suggests that there are some differences in the mechanisms of antinociceptive action of the narcotic analgesics and β-HHC. Tolerance also develops to the increased catecholamine synthesis produced by Δ^9-THC (Maitre *et al.*, 1972). However it is not yet known if tolerance develops to any of the biological effects of β-HHC. The above data suggest that there are both similarities and differences in β-HHC and morphine and their sites of action. Naloxone antagonizes some of the effects of both, but there does not appear to be cross-tolerance between these compounds. Hine *et al.* (1975) reported that Δ^9-THC attenuated the naloxone-precipitated abstinence syndrome in morphine pellet-implanted rats. This finding suggests the possibility that there are common sites of action for both the narcotic analgesics and the behaviorally active cannabinoids in the rat. Alternatively the cannabinoids may interact directly with naloxone. Conversely, Aceto *et al.* (1975) reported that β-HHC did not suppress abstinence in morphine-dependent monkeys. This fact is in agreement with our data demonstrating that cross-tolerance is not present between these compounds in the mouse.

It has been proposed that many of the biological effects of Δ^9-THC are actually due to its primary metabolite, 11-OH-Δ^9-THC (Christensen *et al.*, 1971; Ben-Zvi *et al.*, 1970). While this may be true, the present data suggest that 11-hydroxylation is not necessary for the tail-flick activity and catecholamine effects of the cannabinoids since it is unlikely that β-HHC can be 11-hydroxylated. Wilson and May (1975) also reported that other cannabinoids that cannot be 11-hydroxylated possess analgesic activity. However, they found that the 11-hydroxy metabolites of Δ^9- and Δ^8-THC are more potent than the parent compounds and may be responsible for their analgesic activity. It is apparent though, that 11-hydroxylation is not necessary for many of the actions of the cannabinoids (Wilson and May, 1974).

In summary, these studies with β-HHC indicate that this compound may be a strong analgesic in man with a potency similar to that of morphine. This compound appears to share some characteristics with the narcotics such as antagonism by naloxone but is sufficiently different as not to exhibit cross-tolerance or cross-dependence. It is not known as of now whether this compound can produce primary physical dependence, but studies on this point are in progress. The studies reported here do indicate that 9-*nor*-9β-hydroxyhexahydrocannabinol may be a prototype of a useful class of strong analgesics. Furthermore, the data presented in this paper offer further evidence for the hypothesis that activation of catecholamine-containing neurons is involved in the central mediation of the inhibition of the tail-flick response produced by analgesics.

Acknowledgments. The authors are grateful to Drs. Raymond S. Wilson and Everette L. May of the National Institute of Arthritis, Metabolism and Digestive Diseases for the generous supply of the 9-*nor*-hydroxyhexahydrocannabinols; the National Institute on Drug Abuse for the supply of Δ^9-tetrahydrocannabinol, to Endo Laboratories, Inc. for the supply of naloxone; and to General Aniline and Film Corp. for the generous supply of Emulphor EL-620 which was used as a vehicle for the cannabinoids.

References

Aceto, M. D., Harris, L. S., Dewey, W. L. and Balster, R. L.: Dependence studies of new compounds in the rhesus monkey. Reported to the Committee on Problems of Drug Dependence of the National Academy of Sciences, Washington, D. C., pp. 729–772, 1975.

Archer, S., Harris, L. S., Albertson, N. F. and Pierson, A. K.: Narcotic antagonists as analgesics. Advan. Chem. Ser. 45: 162–169, 1964.

Ben-Zvi, S., Mechoulam, R. and Burstein, S. H.: Indentification through synthesis of an active

Δ^1(6)-tetrahydrocannabinol metabolite. J. Amer. Chem. Soc. 92: 3468-3470, 1970.

BICHER, H. I., AND MECHOULAM, R.: Pharmacological effects of two active constituents of marihuana. Arch. Int. Pharmacodyn. Thér. 172: 24-31, 1968.

BLOOM, A. S., DEWEY, W. L., HARRIS, L. S. AND BROSIUS, K. K.: The correlation between antinociceptive activity of narcotics and their antagonists as measured in the mouse tail-flick test and increased synthesis of brain catecholamines. J. Pharmacol. Exp. Ther. 198: 33-41, 1976a.

BLOOM, A. S., JOHNSON, K. M., BOWMAN, F. J. AND DEWEY, W. L.: Correlation between the effects of several cannabinoids on catecholamine biosynthesis, body temperature and behavior. Fed. Proc. 35: 506, 1976b.

BLUMBERG, H., DAYTON, H. B. AND WOLF, P. S.: Counteraction of narcotic antagonist analgesics by the narcotic antagonist naloxone. Proc. Soc. Exp. Biol. Med. 123: 755-758, 1966.

BUXBAUM, D. M.: Analgesic activity of Δ^9-tetrahydrocannabinol in the rat and mouse. Psychopharmacologia 24: 275-280, 1972.

CHESHER, G. B., DAHL, C. J., EVERINGHAM, M., JACKSON, D. M., MARCHANT-WILLIAMS, H. AND STARMER, G. A.: The effect of cannabinoids on intestinal motility and their antinociceptive effect in mice. Brit. J. Pharmacol. 49: 588-594, 1973.

CHRISTENSEN, H. D., FREUDENTHAL, R. I., GIDLEY, J. T., ROSENFELD, R., BOEGLI, G., TESTINO, L., BRINE, D. R., PITT, C. G. AND WALL, M. E.: Activity of Δ^8- and Δ^9-tetrahydrocannabinol and related compounds in the mouse. Science (Washington) 172: 165-167, 1971.

CLOUET, D. H., AND RATNER, M.: Catecholamine biosynthesis in brains of rats treated with morphine. Science (Washington) 168: 854-856, 1970.

CRADOCK, J. C., DAVIGNON, J. P., LITTERST, C. L. AND GUARINO, A. M.: An intervenous formulation of Δ^9-tetrahydrocannabinol using a non-ionic surfactant. J. Pharm. Pharmacol. 25: 345, 1973.

D'AMOUR, F. E. AND SMITH, D. L.: A method for determining loss of pain sensations. J. Pharmacol. Exp. Ther. 72: 74-79, 1941.

DEWEY, W. L., HARRIS, L. S., HOWES, J. F., KENNEDY, J. S., GRANCHELLI, F. E., PARS, H. G. AND RAZDAN, R. K.: Pharmacology of some marihuana constituents and two hetrocyclic analogues. Nature (London) 266: 1265-1267, 1970a.

DEWEY, W. L., HARRIS, L. S., HOWES, J. F. AND NUITE, J. A.: The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J. Pharmacol. Exp. Ther. 175: 435-442, 1970b.

DEWEY, W. L., HARRIS, L. S. AND KENNEDY, J. S.: Some pharmacological and toxicological effects of 1-trans-Δ^8 and 1-trans-Δ^9-tetrahydrocannabinol in laboratory rodents. Arch. Int. Pharmacodyn. Thér. 196: 133-145, 1972.

DEWEY, W. L., SNYDER, J. W., HARRIS, L. S. AND HOWES, J. F.: The effects of narcotics and narcotic antagonists on the tail-flick response in spinal mice. J. Pharm. Pharmacol. 21: 548-550, 1969.

GALLAGER, D. W., SANDERS-BUSH, E. AND SULSER, F.: Dissociation between behavioral effects and changes in metabolism of cerebral serotonin following Δ^9-tetrahydrocannabinol. Psychopharmacologia 26: 337-345, 1972.

HARRIS, L. S. AND PIERSON, A. K.: Some narcotic antagonists in the benzomorphan series. J. Pharmacol. Exp. Ther. 143: 141-148, 1964.

HINE, B., FRIEDMAN, E., TORRELIO, M. AND GERSHON, S.: Morphine dependent rats. Blockade of precipitated abstinence by tetrahydrocannabinol. Science (Washington) 187: 443-445, 1975.

LITCHFIELD, J. T., JR. AND WILCOXON, F.: A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113, 1949.

MAITRE, L., BAUMANN, P. A. AND DELINI-STULA, A.: Neurochemical tolerance to cannabinols. *In* Cannabis and Its Derivatives, Pharmacology and Experimental Psychology, ed. by W. D. M. Paton and J. Crown, Oxford University Press, London, 1972.

MAITRE, L., STAEHELIN, M. AND BEIN, H. J.: Effect of an extract of cannabis and of some cannabinols on catecholamine metabolism in rat brain and heart. Ag. Actions 1: 136-143, 1970.

MARTIN, B. R., DEWEY, W. L., ACETO, M., ADAMS, M., EARNHARDT, J. AND CARNEY, J.: A potent antinociceptive cannabinoid which lacks opiate substitution properties in monkeys. Res. Commun. Chem. Pathol. Pharmacol. (in press) 1976.

SEDVALL, G. C., WEISE, U. K. AND LOPIN, I. J.: The rate of norepinephrine synthesis measured *in vivo* during short intervals; influence of adrenergic nerve impulse activity. J. Pharmacol. Exp. Ther. 159: 274-282, 1968.

SHELLENBERGER, M. K. AND GORDON, J. H.: A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas. Ann. Biochem. 39: 356-372, 1971.

SMITH, C. B., SHELDON, M. I., BEDNARCZYK, J. H. AND VILLARREAL, J. E.: Morphine-induced increases in the incorporation of ^{14}C-norepinephrine in the mouse brain: Antagonism by naloxone and tolerance. J. Pharmacol. Exp. Ther. 180: 547-557, 1972.

SOFIA, R. D., NALEPA, S. D., HARAKAL, J. J. AND VASSAR, H. B.: Anti-edema and analgesic properties of Δ^9-tetrahydrocannabinol (THC). J. Pharmacol. Exp. Ther. 186: 646-655, 1973.

WAALKES, T. B., AND UDENFRIEND, S.: Fluorometric method for estimation of tyrosine in plasma and tissues. J. Lab. Clin. Med. 50: 733-736, 1957.

WAY, E. L., LOH, H. H. AND SHEN, F.: Simultaneous quantative assessment of morphine tolerance and physical dependence. J. Pharmacol. Exp. Ther. 167: 1-8, 1969.

WEINER, N. AND RABADJIJA, M.: The effect of nerve stimulation on the synthesis and metabolism of norepinephrine in the isolated guinea-pig hypogastric nerve-vas deferens preparation. J. Pharmacol. Exp. Ther. 160: 61-71, 1968.

WILSON, R. S. AND MAY, E. L.: 9-*nor*-Δ^8-Tetrahydrocannabinol, a cannabinoid of metabolic interest. J. Med. Chem. 17: 475-476, 1974.

WILSON, R. S. AND MAY, E. L.: Analgesic properties of the tetrahydrocannabinols, their metabolites and analogs. J. Med. Chem. 18: 700-703, 1975.

WILSON, R. S., MAY, E. L., MARTIN, B. R. AND DEWEY, W. L.: The 9-*nor*-9-Hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with tetrahydrocannabinols. J. Med. Chem. 19: 1165-1167, 1976.

WINER, B.: Statistical Principles in Experimental Design, McGraw-Hill Book Company, New York, 1972.

ZIGMOND, M. J. AND WURTMAN, R. J.: Daily rhythm in the accumulation of brain catecholamines from circulating ^{3}H-tyrosine. J. Pharmacol. Exp. Ther. 172: 416-422, 1970.