Pharmacology Biochemistry & Behavior, Vol. 21, pp. 641–644, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Comparative Effects of Tetrahydrocannabinol on Psychostimulant-Induced Behaviors[1]

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Received 30 January 1984

MOSS, D. E., G. F. KOOB, S. B. McMASTER AND D. S. JANOWSKY. *Comparative effects of tetrahydrocannabinol on psychostimulant-induced behaviors.* PHARMACOL BIOCHEM BEHAV **21**(4) 641–644, 1984.—The behavioral effects of delta-9-tetrahydrocannabinol (THC), one of the major psychoactive cannabinoids in marijuana, were tested in two models of psychostimulant-induced behaviors in rats (locomotor behavior and stereotyped gnawing) induced by amphetamine (AMPH) and methylphenidate (MEPH). Pretreatment with THC (10 mg/kg gavage) almost doubled the amount of AMPH-induced gnawing but produced no effect on AMPH-induced locomotor behavior. In contrast to AMPH, THC produced no direct effect on MEPH-induced gnawing but caused a strong suppression of MEPH-induced locomotor activity. In addition, there was no additional interaction between THC and reserpine as measured by suppression of MEPH-induced gnawing. This result was unexpected in view of the powerful interaction between THC and reserpine reported previously. Because of the clear THC-induced dissociation of the behavioral effects of these two psychostimulants (AMPH and MEPH), our working hypothesis is that THC affects motor behaviors by some non-dopaminergic mechanism.

Tetrahydrocannabinol THC Marijuana Psychostimulant Amphetamine
Methylphenidate Locomotor behavior Stereotyped gnawing Reserpine

THE cannabinoids have been reported to produce dramatic and clinically interesting interactions with certain other drugs. For example, tetrahydrocannabinol (THC, one of the principal psychoactive cannabinoids in marijuana) has been reported to produce up to a 25 fold potentiation of reserpine- and haloperidol-induced hypokinesia [14,15]. Similar results have been obtained with levonantradol, a synthetic analog of THC [15]. These interactions are potential keys to the enigma of the mechanism(s) by which the cannabinoids affect behavior.

The finding that THC produces a large behavioral effect in the reserpine syndrome presents an opportunity to study the effect of THC on other related behaviors. In fact, because certain psychostimulant-induced behaviors are controlled, at least in part, by the same neuroanatomical areas and dopamine systems affected by reserpine [1], THC should also produce powerful effects on these behaviors. However, recent reviews of interactions between cannabinoids and a wide variety of psychostimulants and behavioral measures (hyperthermia, tachycardia, conditioning, locomotion, etc.) in rodents report contradictory and variable results depending upon species, housing, test conditions and other factors [3, 4, 11, 17, 19].

The purpose of the present experiments was, therefore, to compare the effect of THC on psychostimulant behaviors induced by methylphenidate and amphetamine. Locomotor behavior and stereotyped gnawing were selected for study because they are controlled to a great extent by dopaminergic functions in well defined areas of the central nervous system [1, 6, 8, 21]. Lastly, psychostimulant-induced behaviors (locomotor behavior and stereotyped gnawing) are easily and reliably measured.

The general hypothesis was that THC, if affecting behavior through some general dopaminergic mechanism(s), would alter predictably psychostimulant-induced behaviors. Therefore, the effect of THC was assessed on amphetamine- and

[1]This research was supported in part by Grant No. RR08012 funded by the National Institute of Mental Health and the MBRS Program of the Division of Research Resources of NIH and Alcohol Research Center Grant AA 03504.

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methylphenidate-induced locomotor behaviors (Experiment I) and stereotyped gnawing (Experiment II). Furthermore, as a special assessment of the interaction reported earlier, the effect of THC on reserpine-induced suppression of methylphenidate-induced gnawing was also studied (Experiment II).

METHOD

Experiment I—Locomotor Behavior

The subjects were 32 male albino rats (250–300 g), maintained in a temperature and light controlled environment (12 hours light/12 hours dark) in the animal colony at the Salk Institute. Locomotor activity was measured in a bank of 16 wire cages 20×25×36 cm each with two horizontal infrared photocell beams across the long axis 2 cm above the floor. Total photocell beam interruptions were recorded every 10 min from automatic counters located in an adjacent room.

All animals were pretreated by *gavage* with either 10.0 mg/kg delta-9-THC or the 5% ethanol v/v olive oil vehicle. The delta-9-THC was supplied by the Research Technology Branch of the National Institute on Drug Abuse as 200 mg/ml in absolute ethanol. The THC was prepared for *gavage* by diluting this material 1:20 into olive oil under N_2 so that the final concentration was 10 mg THC/ml. *Gavage* administration of the THC was selected to be parallel to the procedure used by Moss *et al.* [14] and to allow for biological absorption, metabolism and distribution of this insoluble resin. Both methylphenidate HCl and D-amphetamine sulfate were prepared in 0.9% physiological saline for injection SC in a 1 ml/kg volume.

Sixty min following pretreatment with THC or vehicle, the animals were placed in the photocell cages and allowed to habituate to the experimental situation for an additional 60 min. Immediately after the habituation period (2 hours after THC or vehicle treatment) the rats were injected with either 3 mg/kg amphetamine or 10 mg/kg methylphenidate and locomotor activity was then recorded in 10 min intervals for the next 3 hours. These doses were selected for detailed study because they are the lowest doses that reliably produced a large increase in locomotor behavior in control animals. Using this paradigm, the effect of THC could be monitored from 2 to 5 hours from THC or vehicle treatment which is the time period during which THC produced the greatest potentiation of reserpine-induced hypokinesia [14].

The data were analyzed according to the procedure of Winer [23] for two factor experiments (i.e., drug condition vs. time) with repeated measures on one factor (time).

Experiment II—Gnawing Behavior

The subjects were 228 female Sprague-Dawley albino rats maintained with ad lib food and water in a light (12 hours light/12 hours dark) and temperature controlled environment in the animal colony at the University of Texas at El Paso (UTEP). Female rats were selected because they show more reliable methylphenidate-induced stereotyped gnawing at considerably lower doses (30 to 40 mg/kg) than do males (60 mg/kg or more).

Stereotyped gnawing was recorded according to the simple automated procedure developed by Moss *et al.* [13] at UTEP. This procedure is based on the simple observation that stereotyped gnawing includes grasping the object being gnawed and pulling up vigorously. Because of this, gnawing can be easily and reliably measured by connecting a microswitch to a piece of hardware cloth (wire mesh 12.5 cm square) anchored loosely to the floor of the gnawing chamber (23 cm square) and counting the number of times the microswitches are operated [13].

All animals were pretreated by *gavage* with either THC (10 mg/kg) or vehicle control 3 hours prior to the behavioral tests in accordance with the procedures described in Experiment I. This dose of THC and the interval at which it was administered before behavioral tests is based upon the earlier observations on THC potentiation of reserpine-induced hypokinesia and corresponds to a time at which a reliable behavioral effect of THC is observed [14]. Animals pretreated with reserpine received the doses indicated in the figures IP 18–24 hours before the behavioral tests to allow ample time for development of a stable reserpine-induced depletion of the monoamines. The reserpine was prepared for injection by diluting the commercial preparation of 2.5 mg/ml so that the amount injected was 1 ml/kg. The tests for stereotypy were initiated by injection of methylphenidate HCl (30 mg/kg IP) or D-amphetamine sulfate (10 mg/kg SC). These doses were selected because they are the lowest doses that produced gnawing in most of the control animals. Immediately thereafter, the animals were placed in the test chambers for a two hour test period. The different routes of administration (IP vs. SC) for the two different drugs were used to produce the most reliable effect with each individual drug. Statistical comparisons were computed by analysis of variance [23].

RESULTS

Experiment I—Locomotor Behavior

In general, as might be expected from a nonspecific sedative effect, THC produced a significant reduction of spontaneous motor activity recorded during the habituation period. There was, however, no main effect of THC on amphetamine-induced activity, $F(1,15)=0.047$, NS, and no THC interaction with time, $F(17,238)=0.461$, NS, although there was a significant change in amphetamine-induced locomotor behavior across the 3 hour test period, $F(17,238)=2.179$, $p<0.01$ (Fig. 1).

In contrast to the result obtained with amphetamine, the effect of THC on methylphenidate-induced locomotor behavior was very clear. THC produced a highly significant reduction in locomotor behavior, $F(1,13)=9.659$, $p<0.01$. There was also a significant effect of time across the 3 hour test period, $F(17,221)=3.307$, $p<0.001$, and a significant interaction between the effect of THC and time, $F(17,221)=2.371$, $p<0.01$ (Fig. 1).

Experiment II—Gnawing Behavior

Figure 2 shows the direct effect of THC (10 mg/kg) and combinations of both THC and reserpine (0.25 and 1.0 mg/kg) on methylphenidate-induced gnawing. As expected from earlier research [18], reserpine had a highly significant suppressive effect on methylphenidate-induced gnawing, $F(2,88)=8.146$, $p<0.01$. A direct comparison of the two groups receiving 1.0 mg/kg reserpine showed that the THC/reserpine group showed significantly less gnawing than the reserpine only group, $F(1,30)=4.569$, $p<0.05$. There were no other significant main effects or interactions.

Figure 3 shows the effect of THC on amphetamine-induced gnawing with and without additional pretreatment by 1.0 mg/kg reserpine. In accordance with the classic report

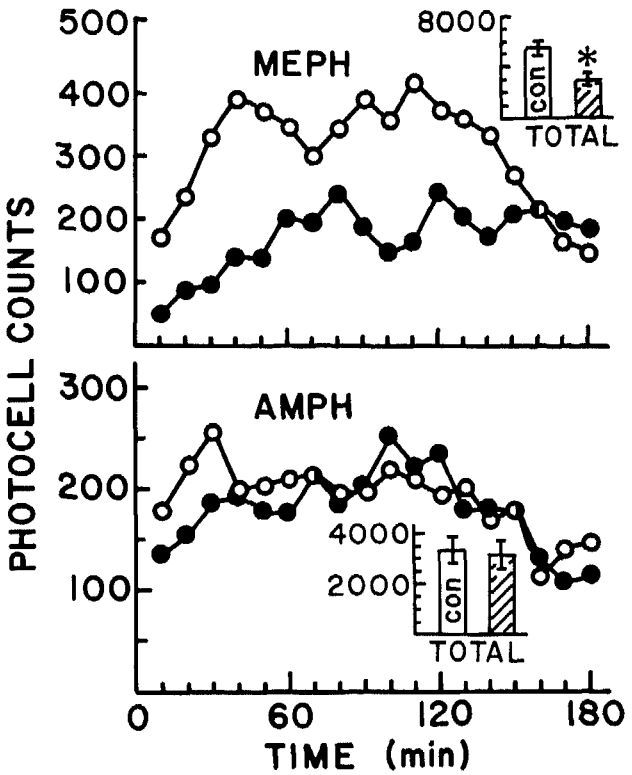

FIG. 1. The effect of THC (10 mg/kg) on amphetamine-induced (3 mg/kg) and methylphenidate-induced (10 mg/kg) locomotor behavior in blocks of 10 min following psychostimulant administration. The mean activity level of vehicle treated control animals is represented by open circles whereas the mean activity level of THC treated animals is represented by filled circles. There were 8 animals in each group. The insert shows total activity for the 3 hour test period (shaded bars show THC groups).

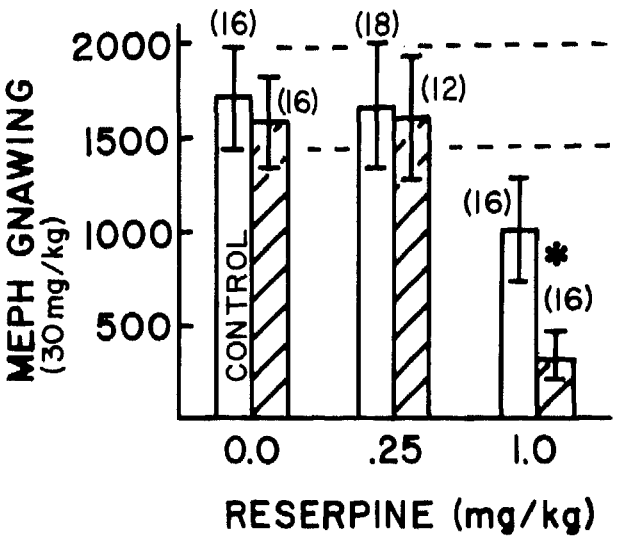

FIG. 2. The effect of THC (10 mg/kg) on methylphenidate-induced gnawing at different doses of reserpine. The bars represent the group means (open bars represent control, shaded bars THC treated) and the error bars are one SEM. The number of animals in each group is shown in parentheses.

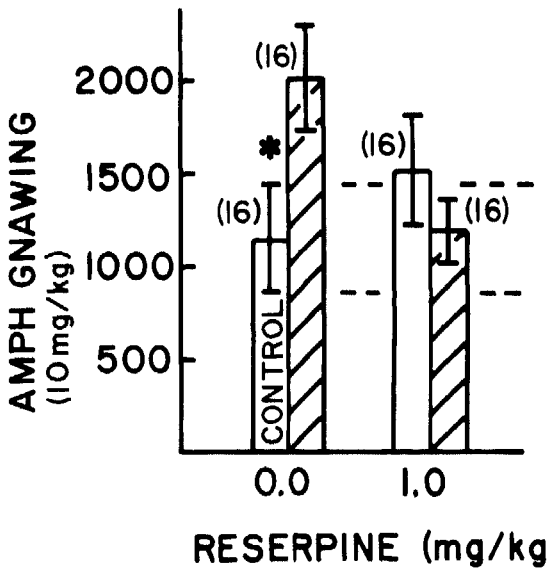

FIG. 3. The effect of THC (10 mg/kg) on amphetamine-induced gnawing with and without additional pretreatment with reserpine. The mean performance of control animals (no THC) is represented by open bars while the performance of THC pretreated animals is shown by shaded bars. The number of animals in each group is shown in parentheses and the error bars represent one SEM.

by Scheel-Kruger [18], reserpine alone had virtually no effect on amphetamine-induced gnawing. In addition, the main effect of THC alone was also not significant. Surprisingly, however, the interaction between THC and reserpine as shown on amphetamine-induced gnawing was significant, $F(1,60)=4.871$, $p<0.05$. The interaction between THC and reserpine shown in Fig. 3 is caused by significant THC potentiation of amphetamine-induced gnawing in the absence of reserpine, $F(1,30)=4.443$, $p<0.05$, and the apparent suppression of this effect by reserpine.

DISCUSSION

The general hypothesis was that THC, if it affected behavior through some general effect on dopaminergic mechanism(s), would have a uniform and a predictable effect on psychostimulant-induced behaviors independent of the indirect sympathomimetic used. The results observed in Experiments I and II, however, presented some interesting contrasts. Contrary to the strong suppression of methylphenidate-induced locomotor behavior, pretreatment with THC produced absolutely no change in amphetamine-induced locomotion. On the other hand, THC produced no effect on methylphenidate-induced gnawing but it enhanced (almost doubled) amphetamine-induced gnawing. Furthermore, in spite of the powerful interaction between THC and reserpine reported earlier [14], similar interactions were not observed in these experiments. The failure to observe an interaction between THC and reserpine in the special experiment on methylphenidate-induced gnawing is particularly interesting because methylphenidate and reserpine apparently act on the same vesicle "pool" of neurotransmitter [18].

The failure to observe a similar effect of THC on methylphenidate- and amphetamine-induced locomotion might be

explained by appearance of a stereotypy which interfered with locomotor behavior [16]. However, no interfering stereotypy was observed during the locomotor tests and similar THC effects were observed at lower doses of psychostimulants (unpublished data). In addition, the lack of a uniform interaction between THC and the two psychostimulants tested cannot be explained by a metabolic interaction with only one of the drugs because THC affected only one behavior induced by each drug. THC suppressed as a behavior organized largely by the mesolimbic dopamine system [1,8] (methylphenidate locomotion) and enhanced a behavior organized largely by the nigrostriatal dopamine system [1,8] (amphetamine stereotypy). The alternative behaviors induced by each drug were unaffected.

The absence of a uniform and predictable effect of THC on dopamine-dependent behaviors (locomotor behavior and stereotyped gnawing) did not support the hypothesis that THC produces its behavioral effects (i.e., potentiation of reserpine-induced hypokinesia) by some direct dopaminergic mechanism within the mesolimbic and/or nigrostriatal systems. Although contrasts of this type are unusual, previous research has shown several pharmacological differences between the nigrostriatal and mesolimbic dopamine systems [2]. Our working hypothesis is that THC produces its behavioral effect through some neurotransmitter or neurotransmitters that interact with the major dopamine systems.

Continued research into understanding a psychopharmacological mechanism of THC action on behavior will not only help explain the enigma of the mood elevating effect of THC but it will also explain the origin of interactions that have significant clinical implications in understanding mental disorders. For example, there are several clinical reports which suggest that cannabis use can induce or exacerbate psychotic disorders [7] which are thought to involve the mesolimbic and mesocortical dopamine systems [5,12]. Furthermore, THC potentiation of hypokinesia may have clinical relevance in improving control of hyperkinetic motor disorders such as tardive dyskinesia or Huntington's disease wherein such drugs as reserpine and haloperidol have demonstrated clinical efficacy [9, 10, 20, 22].

ACKNOWLEDGEMENT

We also thank Hugh Petit for excellent technical assistance.

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