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PROLONGED CNS HYPEREXCITABILITY IN MICE AFTER A SINGLE EXPOSURE TO DELTA-9-TETRAHYDROCANNABINOL

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Summary—A single exposure to delta-9-tetrahydrocannabinol (THC) resulted in a "rebound" hyperexcitability in the CNS in mice, which was assessed in terms of the susceptibility of the CNS to electrically-induced convulsions. The magnitude of the hyperexcitability was dose-related (25–150 mg/kg, i.p.), as measured 24 hr after treatment. The time-course study of the effect indicated a peak-effect at 24 hr after administration of the drug, with a duration of the effect for as long as 196 hr. The time course of the rebound hyperexcitability to THC was compared to that for phenobarbital, which peaked at 48 hr after administration of the drug and returned to the control value by 96 hr. Tolerance developed rapidly to the motor-toxic effect of THC, but after 23 days of daily treatment there was no evidence of tolerance to the rebound hyperexcitability. The functional significance of the hyperexcitable state was assessed in two tests: electrical kindling to minimal convulsions was enhanced, even when the kindling procedure was initiated 120 hr after exposure to the drug; and the anticonvulsant activity of phenytoin was blocked when mice were treated with the anticonvulsant 96 hr after a single exposure to THC. The results suggest that the rebound response from a single exposure to THC represents a functionally significant prolonged increase in excitability of the CNS.

Key words: delta-9-tetrahydrocannabinol, withdrawal hyperexcitability, toxicity, drug interactions.

The complexity of the pharmacology of delta-9-tetrahydrocannabinol (THC) is illustrated by the diversity of the reported effects which include both central depression and excitation (Truitt and Braude, 1974; Paton, 1975). Excitatory effects have been described in a variety of test systems and the effects range from convulsions in conscious animals (Consroe, Jones, Laird and Reinking, 1976; Feeney, Spiker and Weiss, 1976; Chiu, Olsen, Borys, Karler and Turkanis, 1979; Turkanis and Karler, 1982) to an increase in excitatory postsynaptic potentials in spinal motoneurons (Turkanis and Karler, 1983). Despite these observations, THC is generally considered to be a depressant drug because, in sufficiently large doses, central depression, such as motor toxicity, is one of its most conspicuous effects. On the assumption that THC is a depressant, its potential for producing a withdrawal or "rebound" hyperexcitability following subacute treatment with anticonvulsant doses was investigated earlier (Karler and Turkanis, 1980). Increases in excitability were seen 24 hr after withdrawal, as demonstrated by decreases in convulsive thresholds. The results of the present study show that the rebound hyperexcitability is not limited to instances of subacute or repeated exposure to the drug, because a functionally significant increase in excitability can occur after a single dose of THC.

METHODS

Experimental animals and preparations of drugs

The experiments were conducted on 5–6-week-old male CF-1 mice (25–30 g). Delta-9-tetrahydrocannabinol and phenytoin were dispersed in isotonic sodium-chloride solution with the use of Tween 80 and ultrasound, as previously described (Turkanis, Cely, Olsen and Karler, 1974); the preparations of drug and vehicle contained about 3% Tween 80. Sodium phenobarbital was dissolved in its vehicle, isotonic sodium-chloride solution; all preparations of drug and vehicle were administered intraperitoneally.

Experimental procedures and data processing

Central excitability was assessed by 6 and 60 Hz convulsive threshold tests (Karler, Cely and Turkanis, 1974). The electrical stimuli were applied by conventional silver corneal electrodes and the endpoint was a minimal convulsion which was defined as clonus of the jaw and/or front-limb. For 6 Hz stimulation, a constant voltage stimulator (Wahlquist Instrument Co.) was used to generate a 3 sec train of 200 msec rectangular monophasic pulses. For 60 Hz stimulation, a constant-current stimulator (Wahlquist Instrument Co.) was used to generate sinusoidal stimuli of 2 sec duration (Sangdee, Turkanis and Karler, 1982).

Central nervous system excitability was quantified with the use of three measures of susceptibility to electrically-induced convulsions. One measure was the median-convulsive threshold: in order to obtain these values, the intensity of the electroshock stimulus was varied according to the "staircase" method (Finney, 1971), and current- or voltage-effect re-

lationships for minimal convulsions in the 60 or 6 Hz tests were determined. From these curves, the median-convulsive threshold values and their 95% confidence limits were calculated and a relative potency test was used to identify statistically significant differences between control and treatment values ($P \leq 0.05$; Litchfield and Wilcoxon, 1949). Each curve represents the results obtained from 40 to 45 mice. A second measure of CNS excitability was the mean-convulsive threshold (Karler and Turkanis, 1981): in order to obtain these values, the voltage of the 6 Hz stimulus was increased in a step-wise fashion at 5 min intervals until each mouse exhibited a minimal convulsion. A third measure of CNS excitability was the percentage of animals that convulsed in response to a constant electroshock stimulus: in each experiment all of the drug- and vehicle-treated groups of mice received the same electrical stimulus and the percentage of animals convulsing was determined for each group. The parameters for electrical stimulation for each experiment are given in the legends of Figs 2–6. The percentage measurements were used to define the time-course of changes in CNS excitability caused by a single exposure to THC. In these time-course experiments, a different group of 25 mice was used at each test time. In general, the time courses obtained from drug-treated mice were compared statistically to those from controls by a Chi-square test ($P < 0.05$; Snedecor and Cochran, 1967).

In the electrical kindling experiments, mice were given one treatment of THC and the kindling procedure was initiated 24 and 120 hr later during the period of hyperexcitability. Kindling was produced by the corneal administration of 60 Hz, 2 sec stimuli that caused few or no minimal convulsions during the initial test period (Sangdee *et al.*, 1982). After a majority of animals had been kindled to minimal convulsions, electrical stimulation was withheld for 15 days; then, the animals were re-challenged with the kindling stimulus to determine the persistence of this convulsive phenomenon. The time-courses for the development of kindling in the control and treatment groups were compared statistically by the many-one rank statistic ($P < 0.05$; Steel, 1959).

Motor toxicity was determined by a bar-walk test, as previously described (Karler *et al.*, 1974). Briefly, if a mouse was unable to walk a 49 × 1.3 cm steel rod in three attempts in a 2 min period, it was considered to have impaired motor function. Because some untreated animals were unable to perform this task, a preliminary test was included in order to screen out such animals. The bar-walk tests were carried out at the time of the peak-effect of THC, that is, at 2 hr (Karler *et al.*, 1974).

RESULTS

Figure 1(A and B) illustrates the current- and voltage-effect relationships for minimal convulsions evoked 24 hr after one treatment with THC. For the

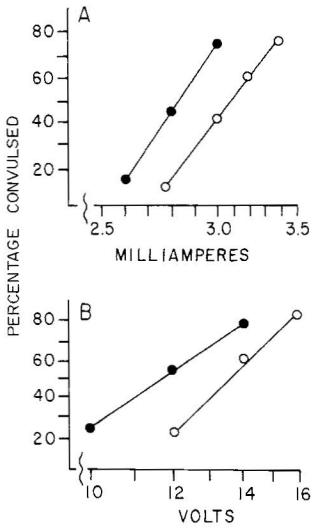

Fig. 1. Thresholds for minimal convulsions after withdrawal from a single treatment with THC. The tests were carried out 24 hr after treatment with cannabinoid or vehicle. (●——●) Data from THC-treated mice; (○——○) data from vehicle-treated mice. (A) The 60 Hz EST test: the median-convulsive thresholds and their 95% confidence limits were 3.1 mA (2.9–3.3) after vehicle and 2.8 mA (2.6–3.0) after THC (150 mg/kg, i.p.). Each curve represents the results from 45 animals. (B) The 6 Hz EST test: the median-convulsive thresholds and their 95% confidence limits were 13.6 V (12–15) after vehicle and 11.6 V (10.6–12.6) after THC (100 mg/kg, i.p.). Each curve represents the results from 40 animals. In both (A) and (B), the median-convulsive thresholds from the mice-treated with drug was significantly different from those of the vehicle controls, as determined by a relative potency test ($P \leq 0.05$; Litchfield and Wilcoxon, 1949).

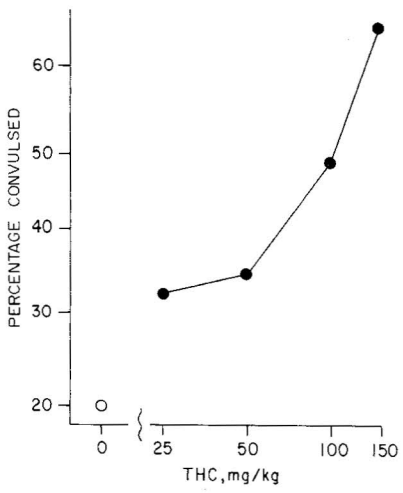

Fig. 2. Susceptibility to minimal convulsions in a 60 Hz electroshock test 24 hr after acute treatment with varying doses of THC. The parameters of the electroshock stimulus administered to each animal were 60 Hz, 2.5 mA and 2 sec; the tests were carried out 24 hr after treatment. (●——●) Data obtained from THC-treated mice; (○——○) data from vehicle-treated controls. Each point represents the results obtained from a group of 25 mice; 5 different groups of mice were used in this experiment. The median-convulsive dose of THC and its 95% confidence limits was 105 mg/kg (i.p.) (69–156; Litchfield and Wilcoxon, 1949).

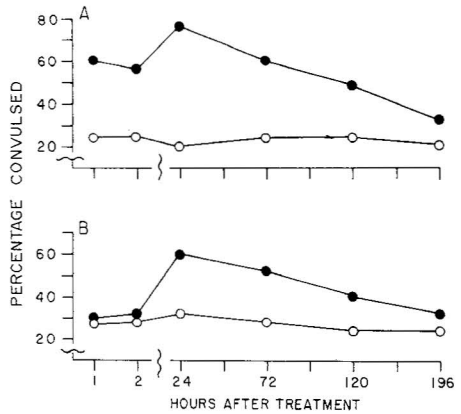

Fig. 3. Time courses of the susceptibility to minimal convulsions in a 60 Hz electroshock test after a single treatment with THC. The parameters of the electroshock stimulus applied to each mouse were 60 Hz, 2.8 mA and 2 sec. Twenty-five mice were used to obtain the data at each test time, and 6 different groups of mice were used to determine each time course. (●——●) Data from THC-treated animals; (○——○) data from vehicle-treated controls. The dose of THC was 100 mg/kg (i.p.) in (A) and 50 mg/kg (i.p.) in (B). In both (A) and (B), the time courses for the effect in drug-treated animals were significantly different from those in vehicle-treated controls, as determined by a Chi-square test ($P < 0.05$; Snedecor and Cochran, 1967).

60 Hz data, shown in Fig. 1A, the median-convulsive thresholds were 3.1 mA for the controls and 2.8 mA for the mice treated with THC; the threshold for the group given the cannabinoid was significantly less than that for the controls. The corresponding findings for the 6 Hz test are shown in Fig. 1B. The median-convulsive thresholds were 13.6 V for the controls and 11.8 V for the animals treated with THC; again, the threshold in the drug-treated mice was significantly less than that of the controls.

The data in Fig. 2 show the percentages of animals exhibiting minimal convulsions in response to a fixed 60 Hz stimulus, administered 24 hr after treatment with varying doses of THC. The findings demonstrate that the rebound hypersusceptibility to convulsions was dose-related. In addition, the results in Figs 1 and 2 illustrate that relatively small changes in threshold produced large increases in the percentage of animals convulsing; therefore, the small changes in threshold observed after treatment with THC are of functional significance.

Figure 3(A and B) show two time courses of the susceptibility to minimal convulsions caused by a consistent 60 Hz stimulus after a single treatment with THC. In the experiment shown in Fig. 3A, the dose of THC was 100 mg/kg and in this instance the acute effect of the drug, as measured 1 and 2 hr after administration, was an increase in CNS excitability, as manifested by an increase in the percentage of animals convulsing, compared with the corresponding vehicle controls. The period of rebound hyperexcitability persisted for up to 196 hr after drug, at which time the value for the animals treated with the drug approximated to that for the controls. In the experiment with THC in Fig. 3B, the mice were treated with only 50 mg/kg compared with the 100 mg/kg used to obtain the data shown in Fig. 3A. The data for the smaller dose shown in Fig. 3B illustrate that the hyperexcitability appearing 24–196 hr after administration of the drug did not depend upon the acute excitatory effect occurring immediately after the drug.

The results shown in Fig. 4 provide additional evidence that the prolonged hyperexcitability beginning 24 hr after administration of THC was not dependent upon an acute excitatory drug effect; the data in this Figure represent CNS excitability measured by the percentage of animals convulsing to a constant 6 Hz stimulus. The acute effect of THC (100 mg/kg) was anticonvulsant, as shown by a marked decrease in the percentage of convulsions at 1 and 2 hr after giving the drug; starting at 24 hr, however, there was again the beginning of a period of prolonged hyperexcitability, as demonstrated by an increase in the percentage of animals convulsing. The findings at 6 Hz clearly illustrate that the hyperexcitability was not simply an extension of the acute effect of the drug but was a true rebound phenomenon. Furthermore, the withdrawal hyperexcitability generalized to at least the two different types of electrical stimulation, namely, 6 and 60 Hz stimuli (Figs 3 and 4). Finally, the period of hyperexcitability measured by the 6 Hz stimulus, as in the 60 Hz experiments, appeared to be relatively long.

Figure 5(A and B) illustrates, for comparative purposes, the time courses of susceptibility to convulsions as measured in the 60 and 6 Hz tests after a

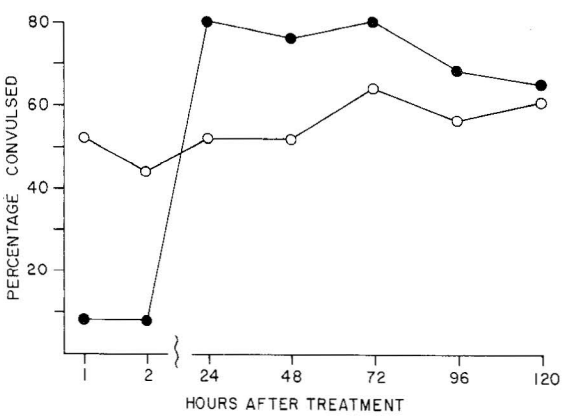

Fig. 4. Time-course of susceptibility to convulsions in a 6 Hz electroshock test after a single treatment with THC. The parameters of the electroshock stimulus applied to each mouse were 6 Hz, 13.0 V and 3 sec. Each data point represents the results from a group of 25 mice and 7 different groups of mice were used to determine each time course. (●——●) Data from THC-treated animals (100 mg kg, i.p.); (○——○) data from vehicle-treated controls. The time course of excitability after treatment with drug was significantly different from that after control treatment, as determined by a Chi-square test ($P < 0.05$; Snedecor and Cochran, 1967).

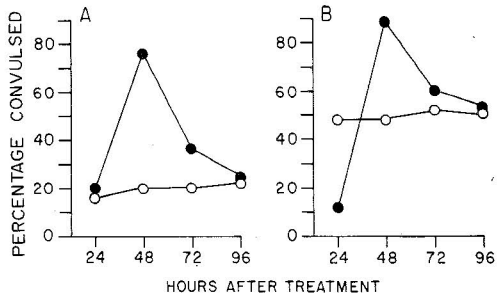

Fig. 5. Time-courses of the susceptibility to minimal convulsions after a single treatment with phenobarbital. Each data point represents the results from a group of 25 mice and 4 different groups of mice were used to determine each time course. (●——●) Data from phenobarbital-treated (100 mg/kg, i.p.) mice; (○——○) data from vehicle-treated controls. (A) The 60 Hz electroshock test: the parameters for the electroshock stimulus administered to each mouse were 60 Hz, 2.6 mA and 2 sec. (B) The 6 Hz electroshock test: the parameters of the electroshock stimulus applied to each mouse were 6 Hz, 13.5 V and 3 sec. In both (A) and (B), the time-course data after administration of drug were significantly different from comparable controls, as determined by a Chi-square test ($P < 0.05$; Snedecor and Cochran, 1967).

single treatment with phenobarbital (100 mg/kg). The 60-Hz data shown in Fig. 5A demonstrate that withdrawal hyperexcitability, as shown by an increased susceptibility to convulsions, at 48 hr and was completely dissipated by 96 hr. The findings at 6 Hz in Fig. 5B are more complex: in this case, the anticonvulsant phenobarbital at 24 hr was anticonvulsant but at 48 hr was proconvulsant; again, the CNS excitability returned to control values within 96 hr.

Figure 6 (A and B) presents the results of repeated daily administration of THC (100 mg/kg) on two effects, motor toxicity and rebound hyperexcitability. In these experiments, animals were injected daily with THC and, in the case of motor toxicity (Fig. 6A), repeated exposure to the cannabinoid caused the rapid development of tolerance. In contrast, the findings of the study of the influence of daily treatment with cannabinoid on the 24-hr rebound hyperexcitability (Fig. 6B) indicate that tolerance did not appear to develop, at least during 22 days of treatment.

The data in Fig. 7 shows the influence of the rebound from a single treatment with THC (100 mg/kg) on the development of electrical kindling to minimal convulsions. The upward shifts in the two kindling time-course curves obtained during withdrawal of the cannabinoid, as compared with the control, indicate a marked enhancement of the phenomenon; for example, in the control, the percentage of mice convulsing on day 8 was 56%; whereas, when the kindling procedure was initiated 24 and 120 hr after giving THC, the comparable values were 84 and 72%, respectively. In other words, there were func-

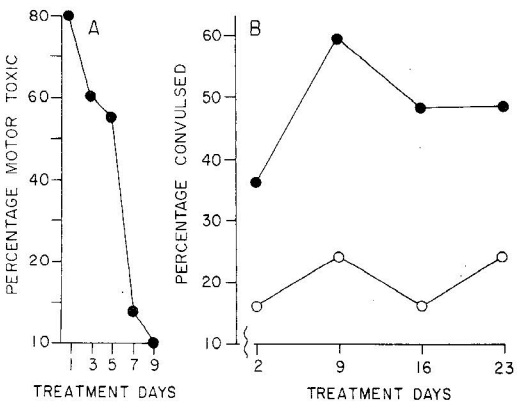

Fig. 6. Influence of repeated treatment with THC on motor toxicity and hypersusceptibility to convulsions. In both (A) and (B), (●——●) data from mice treated daily with THC (100 mg/kg, i.p.); (B) (○——○) data from mice treated daily with vehicle. (A) A group of 25 mice was given daily doses of THC for 9 days and periodically subjected to a bar-walk test at the time of peak-effect of the drug, i.e. 2 hr (Karler *et al.*, 1974). (B) Mice were subjected to the 60 Hz electroshock test 24 hr after giving the drug; the parameters of the electrical stimulus administered to each animal were 60 Hz, 2.6 mA and 2 sec. Each point represents the results from a group of 25 mice; 8 groups of mice were used in this experiment; each groug was shocked only once. The time-course of convulsive activity after repeated exposure to THC was significantly different from that obtained after the vehicle, as determined by a Chi-square test ($P < 0.05$; Snedecor and Cochran, 1967).

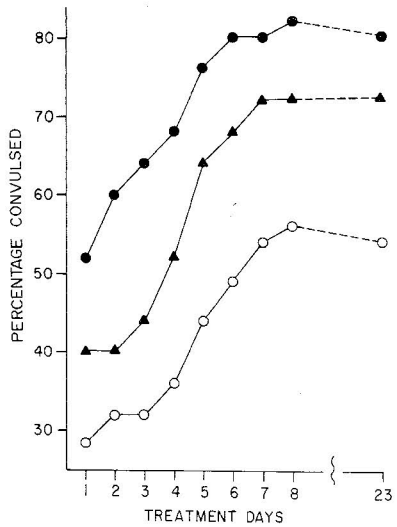

Fig. 7. Time-course of the development of kindling after withdrawal from a single treatment with THC. The kindling stimulus (60 Hz, 2 sec and 3 mA) was administered daily to each mouse for 8 days; then, electrical stimulation was withheld until day 23 at which time the animals were re-challenged with the kindling stimulus. (○——○) Data obtained when the kindling procedure was begun 24 hr after a treatment with vehicle; (●——●) data obtained 24 hr after THC (100 mg/kg, i.p.); (▲——▲) data obtained 120 hr after THC (100 mg/kg, i.p.). Each kindling curve represents the results from 25 mice. The time-course curves obtained during withdrawal from THC were statistically different from the control time-course as determined by the many one rank statistic ($P < 0.05$; Steel, 1959).

tional consequences of the withdrawal, even 120 hr after a single exposure to the drug. In addition, for each group, the percentage of mice convulsing on day 23, 15 days after cessation of the kindling treatment, was essentially identical to that on day 8, the last day of treatment, suggesting that the groups treated with THC reflected a true measure of enhanced kindling (Racine, 1978).

Figure 8 illustrates the results of an interaction study between the anticonvulsant activity of phenytoin and the rebound effect with THC. Figure 8(A) is a dose–effect curve for the anticonvulsant activity of phenytoin in the 6 Hz convulsive threshold test and Figure 8(B) represents the influence of the rebound with THC on the activity of phenytoin (25 mg/kg). In mice with only pretreatment with vehicle, phenytoin raised the threshold from a control value of 15.3–19.7 V. In contrast, 96 hr after a single exposure to THC (100 mg/kg), the median-convulsive threshold after administration of phenytoin was only 16.4 V, which was not significantly different from the control value. In an additional experiment, a similar antagonism of the effect of phenytoin was also observed 24 hr after administration of the cannabinoid.

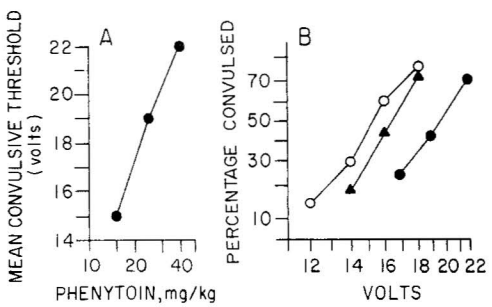

Fig. 8. Influence of withdrawal from a single treatment with THC on the anticonvulsant activity of phenytoin. An increase in the convulsive threshold at 6 Hz was used as a measure of anticonvulsant activity. (A) The relationship between the dose of phenytoin and the mean-convulsive threshold in control mice; each point represents the results from 10 mice. (B) Effect of withdrawal of THC on the anticonvulsant activity of phenytoin on median-convulsive threshold. Each current-effect curve was obtained with 37–41 mice. (○——○) Data from mice that received vehicle 2 and 96 hr prior to the measurements of threshold; the resulting median-convulsive threshold and its 95% confidence limits were 15.3 V (14–17). (●——●) Data from mice that received vehicle 96 hr and phenytoin (25 mg/kg, i.p.) 2 hr prior to measurements of threshold; the median-convulsive threshold and its 95% confidence limits were 19.7 V (18–22). (▲——▲) Data from mice that received THC (100 mg/kg, i.p.) 96 hr and phenytoin (25 mg/kg, i.p.) 2 hr prior to the measurements of threshold; the median-convulsive threshold and its 95% confidence limits were 16.4 V (15–18). The tests were carried out at the time of the peak effect of phenytoin, 2 hr (Turkanis *et al.*, 1974). As determined by a relative potency test ($P < 0.05$; Litchfield and Wilcoxon, 1949), the median-convulsive thresholds from mice treated with vehicle and those from mice treated with phenytoin during withdrawal from THC were not significantly different, but both threshold values were significantly different from those obtained from the vehicle-treated control animals treated with phenytoin.

The results clearly demonstrate that the hyperexcitability due to withdrawal of THC antagonized the depressant activity in the CNS characteristically associated with phenytoin, even 96 hr after a single treatment with THC.

DISCUSSION

The present investigation confirms and extends the observations of earlier work describing an increased susceptibility to electroshock-induced minimal convulsions after withdrawal from THC (Karler and Turkanis, 1980). The previous work, however, differed in several details from that described here. First, the strains of mice were not the same in the two investigations: the original results were obtained with Charles River ICR mice, whereas those in the present work were with Carworth Farms CF-1 mice. The similar response in two different strains suggests that there is at least some generality to the effect. Moreover, in the original research, the hyperexcitability after withdrawal for 24 hr was observed after subacute treatment with THC; only a single treatment was used in the present study, Finally, the two investigations differ in the time at which excitability in the CNS was measured: in the first study, only a withdrawal time of 24 hr was assessed, but in the present work, the complete time course of the hyperexcitability was defined. The major finding of the current experiments was that after a single exposure to THC, increased susceptibility to convulsions began in about 1 day and persisted for as long as 6 days.

The prolonged hypersusceptibility to convulsions appears to be a rebound reaction rather than a result of the long duration of an acute effect. This conclusion is based on the observation that the acute effects, depending upon the specific electroshock test, could be either proconvulsant (60 Hz test) or anticonvulsant (6 Hz test); in either case, a period of hyperexcitability developed 24 hr after administration of drug. Furthermore, 50 mg/kg of THC caused the rebound reaction even in the absence of any detectable acute response to the drug.

The temporal characteristics of the rebound response to phenobarbital were also described in order to provide a basis for comparison of the effect of THC with that of a drug with a well-established ability to produce a rebound hyperexcitability. The data indicate that the effect of THC lasted 2–4 days longer than that elicited by the barbiturate, even though the dose of the barbiturate employed produced anesthesia, which is a much more profound degree of depression of the CNS than that resulting from the doses of THC administered. The results suggest that the relatively long duration of the rebound effect with THC is not the consequence of a greater degree of CNS depression.

Several investigators have previously explored the possibility that cannabis produces withdrawal effects: for instance, two different studies failed to discover

any increase in susceptibility to convulsions caused by pentylenetetrazol in mice and rats after exposure to cannabis (Chesher and Jackson, 1974; Leite and Carlini, 1974); similar results were obtained during the present work with THC. Despite the fact that the rebound effects did not generalize to convulsions caused by pentylenetetrazol withdrawal phenomena with THC were evident in both the present electroshock study as well as in a previous electrophysiological investigation (Pirch, Osterholm, Cohn and Barratt, 1973). In the electrophysiological study, a marked rebound increase in electrocorticographic voltage occurred in rats after chronic exposure to cannabis. The electrocorticographic effect, like those of the present study, was an extremely long-lasting response, persisting for at least 10 days.

The question of whether tolerance develops to the withdrawal hyperexcitability effect was also examined because a prominent feature of the pharmacology of THC is that repeated exposure to the cannabinoid results in the rapid development of tolerance to many of its responses (Paton, 1975). The data presented here show that daily administration of THC resulted in tolerance to motor toxicity; in contrast, there was no indication of tolerance to the hyperexcitability, even after 22 days of treatment. Therefore, the rebound hyperexcitability is one of the few effects of THC to which tolerance does not appear to develop.

In conclusion, the data demonstrate that, after a single exposure to THC, a prolonged hyperexcitability resulted i.e. an increased susceptibility to convulsions began in about 1 day and lasted for up to 6 days. In addition, the hyperexcitability was of functional significance as shown in at least two instances: first, it markedly enhanced the development of kindling which was relatively persistent, i.e. many months (Racine, 1978; Sangdee *et al.*, 1982); consequently, the withdrawal effect of THC may contribute to the production of long-lasting increases in excitability in the CNS. Secondly, the long-lasting hyperexcitability in the CNS after exposure to THC antagonized the anticonvulsant effects of the antiepileptic drug, phenytoin. The significance of these findings in terms of humans who abuse marijuana is not clear, but a rebound hypersusceptibility to convulsions may have contributed to the precipitation of *grand mal* convulsions in an epileptic patient, subsequent to smoking marijuana (Keeler and Reifler, 1967). Furthermore, another potential adverse consequence of abuse of marijuana is that a rebound hyperexcitability in epileptic patients may jeopardize the seizure control they have achieved with such antiepileptic drugs as phenytoin. Regardless of these potential consequences of abuse of marijuana, the results of the present investigation raise the possibility that even a single exposure to marijuana may cause functionally significant prolonged increases in CNS excitability.

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