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Antiepileptic Properties of Δ^9-Tetrahydrocannabinol

J. A. WADA, M. SATO, AND hf. E. CORCORAN [1]

Kinsmen Laboratory of Neurological Research, Faculty of Medicine, University of British Columbia, Vancouver 8, British Columbia, Canada

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Treatment of freely-moving cats with Δ^9-THC, a prominent psychoactive cannabinoid, temporarily reduced the clinical and electrographic seizure activity induced by electrical stimulation of subcortical structures. The antiepileptic effects were not accompanied by gross signs of behavioral toxicity. Seizure activity induced by hypothalamic stimulation seemed to be more sensitive to this drug than that induced by stimulation of dorsomedial nucleus of thalamus.

INTRODUCTION

Available evidence suggests that various cannabinoid drugs possess some antiepileptic properties in terms of amelioration of clinical (behavioral) seizures. For instance, treatment with a dimethylheptylpyran derivative of tetrahydrocannabinol (THC) reduced the incidence of electroconvulsive shock-induced seizure activity in rats (7) ; the same THC derivative was shown to have some therapeutic value for several institutionalized epileptic children (3). Similarly, seizures induced in mice both by electroconvulsive shock (10) and by auditory stimulation (1) have been reduced or blocked by administration of Δ^9-THC, the presumed major psychoactive ingredient of cannabis (S). These studies concentrated on convulsive behavior but did not investigate its possible electrngraphic effects.

On the other hand, electrocorticogram (EEG) of the cat was studied with Ion- doses of Δ^9-THC which increased slow-wave activity, while higher closes increased the fast activity in the EEG of cats (4). Similarly, moderate to high doses of Δ^8-THC, an isomer of Δ^9-THC, induced a rhythmic polyspike discharge in the EEG of sleeping rats (2). Segal and

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Kenny (9) have reported that high doses of Δ^8-THC increase the amount of afterdischarge activity obtained following electrical stimulation of the brain in cats. Yet the same substance in lower doses had no effect upon hippocampal afterdischarge activity induced by electrical stimulation of the reticular formation in rabbits (6). Thus it appears that low doses of cannabis or its constituents produce an effect compatible with the drug's possible ability to serve as an antiepileptic agent, while higher doses produce evidence of CNS activation as well as disturbed electrographic activity.

In order to provide a more complete picture of the drug's antiepileptic activity we have initiated a preliminary examination of the effects of Δ^9-THC on both the clinical convulsive seizures as well as the electrographic seizure discharges induced by electrical stimulation of subcortical structures in awake, freely moving cats.

MATERIALS AND METHODS

Two male and three female cats, weighing 2.7–4.0 kg n-ere used. These cats bore electrodes chronically implanted in the ventromedial hypothalamic region, dorsomedial nucleus of the thalamus, preoptic region, fornix, and frontal cortex. The animals had been used for other experiments and the neural structures had been stimulated intermittently for a period of about 3 mo prior to the present investigation. The electrodes described here were selected from a larger number of electrode placements on the basis of readily produced electrographic and clinical seizures upon their electrical stimulation. The bipolar electrodes n-ere comprised of two strands of a stainless-steel wire 0.19 mm in diameter which n-ere covered with glass at all points but the tips. The stimulation n-as monopolar and consisted of a 5-sec train of 60 Hz sine wave. For threshold determination, the intensity of stimulation was initially set at 0.30 V and was increased by 0.05 V every 3 min until an electrographic seizure discharge associated with some ictal behavioral manifestation was produced. The threshold as determined by this method was found to be stable and readily reproducible over a period of 2-3 mo.

In order to assess both short-term and long-term changes in seizure threshold induced by Δ^9-THC, the threshold voltage level was either measured hourly for a period of 8 hr each day (between 9 AM and 6 PM) or once daily (between 9 AM and noon). The EEG was recorded from frontal cortex as well as from the subcortical structures bearing electrodes, including the site of stimulation. The drug, Δ^9-THC, which was assessed to be 93% pure, was supplied by the Department of National Health and Welfare of Canada and was stored in a refrigerator as a solution in ethanol (200 mg/ml). Shortly before use, a small amount of the drug was further

diluted with propylene glycol and ethanol, and the concentration of the resulting solution was 1 mg of drug dissolved in 1 ml of a vehicle comprised of 9 parts PG to 1 part ethanol (i.e., 1 ml of solution contained 1 mg of Δ^9-THC, 0.9 ml of propylene glycol and 0.1 ml of ethanol). For control injections, a separate vehicle solution of 9 parts propylene glycol to 1 part ethanol was administered at a volume identical to the volume of the drug injection (e.g., 0.25 mg/kg Δ^9-THC = 0.25 ml/kg of vehicle). Most of the observations were made with 0.25 mg/kg Δ^9-THC, but some cats were given up to 0.5 mg/kg. All injections were intraperitoneal. Histological localization of stimulating electrode sites is shown in Fig. 1.

RESULTS

Results are summarized in Table 1 and details are given below.

General Behavioral Effects

One cat (No. 564) began vomiting about 3 min after the drug was injected. Defecation was observed 20 min (No. 564) and 2.5 hr (No. 566) following administration of THC, but these cats also defecated when the vehicle alone was given. All the cats receiving 0.25 mg/kg of Δ^9-THC showed some evidence of behavioral and electrographic drowsiness for about 4 hr after drug administration, with the peak effect occurring at 1–2 hr. The significance of this phenomenon is unclear, however, since a similar trend was noted following administration of vehicle alone. On the other hand, an obvious difference between treatment with Δ^9-THC and with the vehicle can be inferred from the fact that THC-treated cats con-

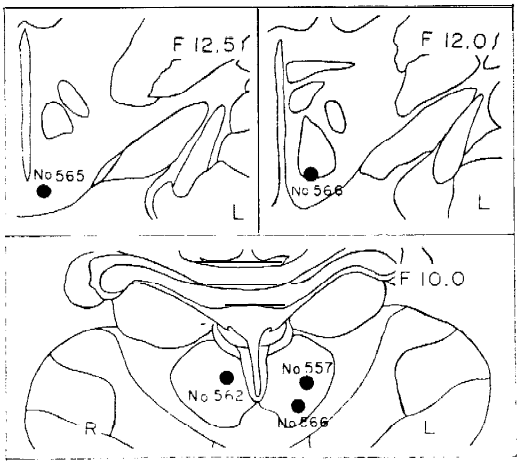

FIG. 1. Localization of stimulating electrode sites.

TABLE 1

SUMMARY OF RESULTS[a]

Investigation	Hourly				24-Hourly	
Cat No.	557	562	566	566	557	565
Site of stimulation	MD	MD	MD	VM	MD	VM
Effect on seizure						
EEG	−	−	+	±	−	+
Clinical	−	±	+	+	−	+
Maximal effect (lir)		1–3	1–2	2–3		

[a] (+) Complete elimination; (±) shortening of duration; (-) no effect; MD, nucleus medialis dorsalis; VM, ventromedial nucleus of hypothalamus.

tinued to respond to loud noises with an excessive behavioral arousal response (e.g., standing up) accompanied by an EEG "arousal" pattern, while the vehicle-treated cats showed a smaller initial response and rapidly habituated. This arousal tendency peaked about 1 hr after THC administration and gradually subsided until the third hour, when a differential response towards arousing stimuli could no longer be detected.

Hourly Examination of Electrographic and Clinical Seizures

Three electrode sites in two cats were tested.

Cat No. 562: *Dorsomedial Thalamus.* In the nondrugged state, electrical stimulation of the right dorsotnedial nucleus of the thalamus at an intensity of 1.05 V produced piloerection and mydriasis during stimulation, followed by twitching of facial muscles lasting 7–10 sec. Subsequently a generalized jerking accompanied by masticatory movements often developed while the animal maintained a standing posture. About 20 sec after the offset of stimulation the cat often ran towards the left, sometimes associated with urination. About 40–45 sec after stimulation offset, the twitching and jerking tended to stop abruptly while the animal remained standing still. Within the subsequent 10-15 sec the cat again became mobile with meowing. Electrographically, the clinical manifestation was coincident with ongoing high-voltage spike or multiple spike and wave discharges, with a marked increase in frequency of spikes during the latter part of the clinical seizure accompanied by urination. The termination of organized seizure discharge was followed by a brief electrographic depression and then the subsequent appearance of continuous high voltage 1-2 cycle/sec slow activity in some areas,

Following administration of 0.25 mg/kg Δ^9-THC, the duration of the seizure was markedly reduced to a maximum of about 25 sec. Clinically,

only if slight head twitching without generalized clonic jerking appeared. Electrographically, there was a considerable reduction both of the duration of spike-wave discharges and of the degree of postical depression and slowing. Administration of a comparable amount of vehicle alone produced a slight reduction in the seizure duration (to approximately 35 sec), but the intensity of the clinical seizure remained unchanged.

Therefore, in this particular cat, Δ^9-THC produced amelioration of the intensity of the clinical seizure as well as a reduction in the duration of both the electrographic and the clinical seizure.

Cut No. 566: Dorsomedial Thalamus. In the nondrugged state, electrical stimulation of the left dorsomedial nucleus of thalamus at an intensity of 0.55 V produced marked pupillary dilatation and piloerection. Following the termination of stimulation, the cat stood immobile and stared ahead. An intermittent forward-running movement then began to alternate with staring, and at times the animal looked around. This seizure, which terminated with meowing, lasted about 30 sec and n-as not followed by postictal exhaustion.

Administration of A"-THC at a dose of 0.25 mg/kg resulted in complete suppression of the clinical and marked suppression of the electrographic seizure at the first hour (Fig. 2, So. 566). At the second and third hours, there was a reduction of the duration of the seizure to a length of approximately 10 sec. The significance of this observation is questionable, however, since a similar reduction in seizure duration has been observed to occur spontaneously in control (nondrug) experiments. On the other hand, there n-as a definite amelioration of the intensity of the clinical seizure even at the second hour after injection. At this time, the cat remained crouched and did not exhibit the intermittent running behavior which was typically displayed in the nondrugged state, Seven hours after administration of Δ^9-THC, a generalized convulsion developed; its significance is uncertain. Administration of the vehicle at a dose of 0.25 mg/kg failed to produce any alteration of either the clinical or the electrograpliic seizure.

Cat No. 566: Ventromedial Region of the Hypothalamus. During electrical stimulation of the left ventromedial area of the hypothalamus at an intensity of 1.25 V, the cat would run ton-ards the left, at times hissing. Following offset of stimulation, the animal remained immobile in an unnatural standing posture and exhibited pupillary dilatation, piloerection and salivation, followed by facial twitching and turning of the head towards the leit. Clonic jerking of the right forepaw combined with head twitching was subsequently observed. The seizure, lasting for 30-50 sec, terminated with the abrupt onset of meowing. Electrographically, the stimulation was followed by semirhythmic 13–15/sec high-voltage sharp activity, which gradually developed into a generalized spike and slow wave complex of

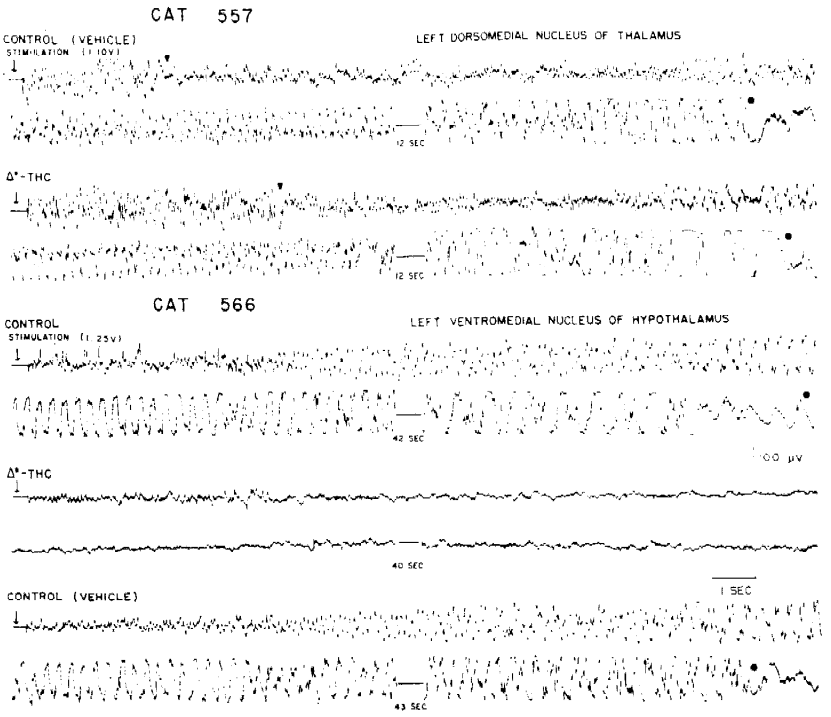

FIG. 2. Cat No. 557: Lack of effect of Δ^9-THC. Note abrupt change of seizure pattern (▼). Cat No. 566: Effective suppression by Δ^9-THC but not its vehicle. ↓ End of stimulation. ● End of behavioral seizure.

about 3–3.5/sec with a progressive recruitment of multiple spike components. There was a profound postictal depression as well as high voltage 1.5 Hz slow waves in some structures.

Administration of 0.25 mg/kg Δ^9-THC caused a complete suppression of electrographic and clinical seizures through the second hour, but by the third hour brief electrographic seizures unaccompanied by behavioral manifestations had returned. Treatment with the vehicle in a comparable dose failed to produce any change in the clinical or EEG pattern.

Daily Examination of Electrographic and Clinical Seizures

One electrode site was examined in each of two cats.

Cat No. 565: Ventromedial Hypothalamus. This cat was stimulated in the left ventromedial nucleus of the hypothalamus at an intensity of 0.6 V. During the latter half of the stimulation the animal turned his head towards the right and twitching of the head and facial muscles was observed. Following stimulation offset, a progressive intensification of pupillary

dilatation, piloerection, salivation and twitching was noted. About 10–15 sec after offset, there appeared a predominantly right-sided twitching of the extremities associated with semiturning towards the right, which gradually developed into generalized clonic jerking. During this generalized jerking, which lasted 42–58 sec, the cat remained standing upright. He maintained an unnatural posture with infrequent twitching of the left facial musculature for about 5 min. at which time "normal" behavior reappeared. Electrographically, there nere generalized high-voltage multiple spike and sharp waves which gradually transformed into high-voltage irregular spike or multiple spike- and-wave complexes in all regions from which recordings were taken.

Both electrographic and clinical seizure manifestations were completely suppressed 1 hr after administration of 0.25 mg/kg Δ^9-THC. As shown in Table 1, complete suppression of epileptiforrn activity by administration of Δ^9-THC was replicated with a second injection on the following day; treatment with vehicle alone, however, had no effect. This dose of Δ^9-THC was ineffective in suppressing the seizure activity elicited by a higher intensity of stimulation, 0.8 V as opposed to 0.6 V.

Cat No. 557: Dorsomedial Thalamus. This animal was stimulated in the left dorsomedial nucleus of the thalamus at two intensities, 1.1 and 1.3 V. Treatment with 0.5 mg/kg Δ^9-THC produced no evidence of suppression either of the clinical (at 1.3 V) or of the electrographic seizure discharge (at 1.05 V) without clinical ictal manifestations—the only completely negative case in this series. Two possible factors may have contributed to this negative finding. First, this cat was the heaviest animal (4 kg) tested in the present experiment and a higher dose might have produced positive results. Second, and perhaps more important, the electrographic seizure pattern evoked in this cat was distinctly different from that of the other subjects. in that the immediate poststimulation discharge pattern always changed abruptly to a low-amplitude multiple sharp discharge which progressively built up into a high-amplitude, full blown electrographic seizure discharge (Fig. 2, No. 557).

This finding suggests that, in this cat at least. distant structures must have been activated secondarily by the primary afterdischarge. Therefore, propagation of seizure discharge into 3 distant set of subcortical structures may be resistant to treatment with Δ^9-THC.

DISCUSSION

The results of the present experiment indicate that the clinical and electrographic epileptiform activity induced by localized electrical stimulation of the cat's brain can be suppressed by administration of Δ^9-THC.

This demonstration confirms and extends the results of previous studies which have shown that clinical convulsive activity induced by electroshock or auditory stimulation in rodents is reduced by treatment with various cannabinoid drugs (1, 10). The importance of taking some measure of the electrographic as well as behavioral seizure activity following treatment with cannabis is indicated by the fact that we noted at least one instance where electrographic abnormalities had reappeared at a time after the injection when clinical convulsions remained suppressed. The antiepileptic activity of Δ^9-THC observed in the present experiment was not absolute, in the sense that an increase in the intensity of the seizure-provoking stimulation n-as capable of counteracting the drug effect. In addition, Δ^9-THC strongly suppressed seizures for only 1–2 hr following injection. This short-term effect is interesting in the light of the fact that THC and metaholites are known to be slowly disposed of and often remain in the plasma and tissues for clays after a single injection (5). These results were obtained at a dose of Δ^9-THC that did not produce marked toxic effects on the general behavior of the cats. In fact, the only reliable behavioral effect we observed was an enhanced arousal response to a loud noise, in conjunction with an impaired tendency to habituate to this noise. More work will be necessary to specify the dose-response relations of the drug's antiepileptic properties, but the results of this preliminary experiment suggest that effective closes need not necessarily produce gross aberrations in the subjects' ongoing behavior.

Negative results were obtained from only one subject in this series. and this cat's stimulating electrode was located in the dorsomedial nucleus of the thalamus. Together with the fact that seizure activity evoked from the hypothalamus seemed more readily disrupted by the drug than that from the thalamus, the lack of effect on epileptiform activity in this cat suggests that different structures in the brain may possess variant sensitivity to the antiepileptic properties of Δ^9-THC. Alternatively, this abrupt change of seizure pattern observed may suggest secondary activation of distant cerebral structures and, therefore! such a distant seizure propagation may be resistant to treatment with Δ^9-THC.

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