ELECTROPHYSIOLOGICAL MECHANISMS OF DELTA-9-TETRAHYDROCANNABINOL'S CONVULSANT ACTIONS*

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I. INTRODUCTION

Delta-9-tetrahydrocannabinol (THC) elicits a complex mixture of central excitatory and depressant properties (1,2). Central excitation, however, represents a major facet of the drug's pharmacological profile, which ranges from vocalization to overt convulsions (3). Its propensity to precipitate frank convulsions is exhibited in various laboratory test systems; for example, in mice, THC lowers the convulsive threshold in the 60-Hz-electroshock threshold and pentylenetetrazol minimal-seizure tests (19), and it also augments the development of electrically and pentylenetetrazol-caused kindling (4). Furthermore, THC produces frank convulsions in cobalt and iron epileptic rats (5,6), in epileptic beagles (7), and in a special strain of rabbits (8). The convulsive properties of THC are well-documented and, accordingly, are potentially important toxicologically.

The purpose of the present work is to identify possible electrphysiological mechanisms of action of the cannabinoid's convulsive effects to further the understanding of its pharmacological properties. The results of electrophysiological investigations carried out at various levels of the central nervous system, ranging from the cerebral cortex to the spinal cord, demonstrate that THC's excitatory effects are discernible at every level studied, even at individual synapses.

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II. METHODS

The techniques used in the present work have been previously described: cobalt epilepsy by Chiu et al. (5); kindled limbic seizures by Smiley et al. (9); monosynaptic spinal reflexes by Esplin (10) and Tramposch et al. (11); cortical evoked responses by Turkanis and Karler (12); and synaptic potentials at spinal motoneurons by Weakly (13) and Kuno and Weakly (14). Spinal-cord studies were carried out with 2.4-3.6 kg male and female cats with their spinal cords severed at the atlanto-occipital junction and their brains destroyed ischemically (10); in all other studies, conscious, unrestrained male Sprague-Dawley rats with electrodes chronically implanted in their brains were used. The drug was prepared by previously published procedures (15) and was given intraperitoneally to rats and intravenously to cats.

III. RESULTS

In conscious rats, THC produces numerous excitatory electrophysiological effects that appear to contribute to its convulsive properties: For instance, the cannabinoid exacerbates a pre-existing epileptic phenomenon; that is, the drug causes a dose-related increase in spontaneously-firing, cortical, focal epileptiform potentials in cobalt epileptic rats (Fig. 1); it is important to note that this excitatory effect occurs

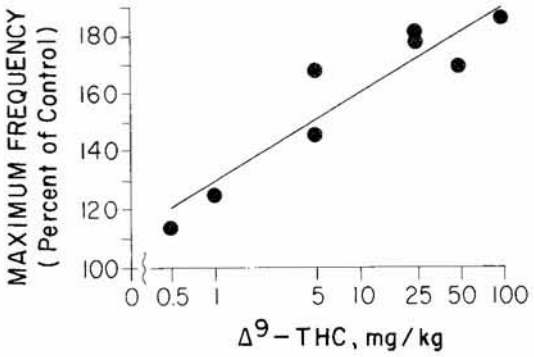

FIGURE 1. Influence of THC on the frequency of spontaneous-firing, focal epileptic potentials at a cobalt focus in the left cortex. Electrocorticographic recordings were made in rats with chronically implanted brain electrodes from a focus in the left parietal cortex. Each point represents the results from a different rat and is the maximum frequency expressed as a percentage of predrug control obtained in each experiment. The line was calculated by least squares regression. Adapted from Ref. 9.

with doses as small as 0.5 mg/kg. Furthermore, the increase in focal epileptiform activity results in the precipitation of convulsions. The drug also enhances neurotransmission between the cerebral cortices, as evidenced by an increase in the amplitude of the electrically caused transcallosal evoked response (Fig. 2). The augmentation of neurotransmission in the corpus callosum, which is important for the transfer of epileptic phenomena (16), may also contribute to the manifestation of the cannabinoid's convulsive effects (12).

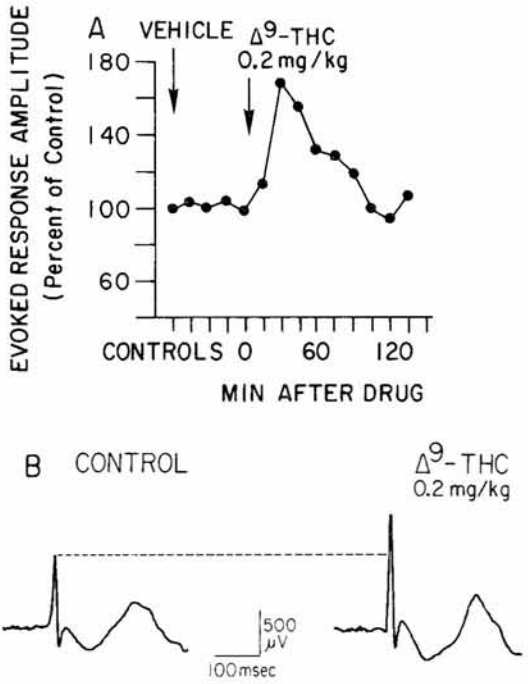

FIGURE 2. Influence of THC on the amplitude of the electrically-caused, transcallosal cortical evoked response. A, the time course represents the results from a rat. The data are amplitudes of the initial phase of averaged evoked responses, and the arrows indicate the time of vehicle or drug administration. Each response is an electronically obtained average of four consecutive potentials obtained 5 sec apart, while the rat was in a sitting position. B, ave. response from a rat before and 30 min after drug. In six rats, THC (0.1-1.0 mg/kg) increased the mean amplitude and its range to 153 (127-208) percent of controls (obtained at the beginning of each experiment). Comparable vehicle values from six rats were 101 (88-111) percent of controls. The two values are statistically different by Mann-Whitney U statistic ($p < 0.05$) (25).

The results of the kindled limbic rat seizure studies show the effects of THC on the after discharge (AD), which is the elecrical manifestation of a seizure (17). In low doses (0.1-1 mg/kg), the cannabinoid prolongs the duration of the AD in the hippocampus and the cerebral cortex but not at the site of focal electrical stimulation in the subiculum (Table I). These data suggest that the drug increases the propagation of the AD selectively in different brain areas. At higher doses (5-15 mg/kg) this selectivity disappears because the AD is markedly prolonged at all three recording sites (Fig. 3). The behavioral consequence of a prolongation of the AD is an increase in the duration of the convulsion. In addition to these effects on electrically induced ADs, THC (0.5-100 mg/kg) produces AD-like bursts of potentials in the cortices of cobalt epileptic rats (Fig. 4); similar results were seen in iron epileptic and control rats (6). Despite the fact that the relationship between drug-induced AD-like bursts and convulsions is unknown, it is possible that the bursts may also contribute to the precipitation of convulsions.

The findings of the spinal reflex investigations with cats are consistent with the cortical and limbic findings: First, THC (0.05-0.15 mg/kg) in the cat increases the amplitude of

TABLE I. Delta-9-THC's Effects on AD Duration of Conscious Rats With Kindled Limbic Seizures

Recording Site	Means and Ranges of Maximum Effects[a]
Left subiculum	96 (75-107)
Right dorsal hippocampus	113 (106-134)[b]
Rigth cerebral cortex	115 (104-119)[b]

[a]Values expressed as percentage of internal vehicle control obtained in each experiment; five experiments were carried out with 0.1-1 mg/kg delta-9-THC. In these studies, the rats had chronically implanted electrodes in their brains; the left subiculum was the site of focal electrical stimulation, and ADs were recorded simultaeously from the subiculum, the hippocampus and the cerebral cortex.

[b]Value is significantly different from that obtained from the left subiculum, as determined by the many-one rank statistic ($p < 0.05$) (26). The mean vehicle control values and their ranges for the hippocampus and cerebral cortex were 92 (86-112) and 93 (86-112), respectively (Ref., Smiley, Turkanis and Karler, unpublised).

the monosynaptic reflex (Fig. 5). Such results support our cortical evoked data indicating that THC increases neurotransmission; furthermore, they implicate the synapse as a site of drug action. Secondly, the drug markedly enhanced posttetanic potentiation (Fig. 6), which is purported to be a mechanism by which seizures spread throughout the CNS (10).

Because the spinal-reflex data suggest that the synapse is a probable site of drug action, the effects of the cannabinoid on synaptic transmission at individual cat spinal motoneurons were also assessed. In this test system, the cannabinoid (0.01-0.1 mg/kg) elicits a consistent excitatory effect; that is, it evokes motoneuron action potentials (Fig. 7). Subsequent experiments demonstrated that in the same dosage range the cannabinoid increases the amplitude of the EPSP (Fig. 8), an effect which could, at least partially, account for the facilitation of the production of motoneuron action potentials. In addition, within the identical dosage range THC partially suppresses inhibitory postsynaptic potentials

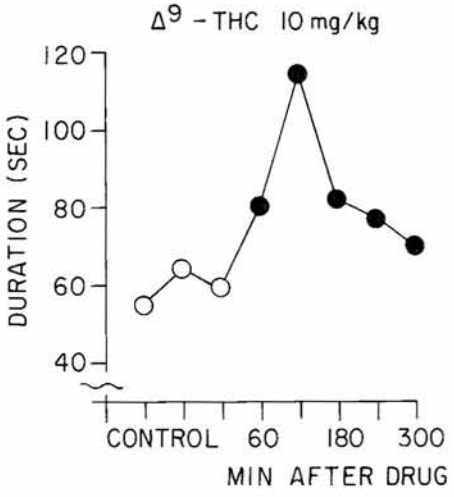

FIGURE 3. Influence of THC on limbic AD duration in a rat with kindled seizures. AD was evoked and recorded from left subiculum of a rat with chronically implanted electrodes. Open circles represent responses after vehicle; closed circles, responses after drug. Nine experiments were carried out with both vehicle and drug: the mean AD duration and its range after vehicle treatment was 93 (85-112) percent of control and after THC (5-15 mg/kg) was 174 (120-344) percent of control. Drug value is significantly different from control as determined by Mann-Whitney U statistic ($p < 0.05$) (25). Adaped from Ref. 3.

(IPSPs); it has, however, a limited efficacy because a 10 mg/kg dose is no more effective than is 0.1 mg/kg (Fig. 9). In short, the drug at a spinal synapse elicits excitation by two different mechanisms; that is, by enhancing EPSPs and by suppressing IPSPs. Since there is a possibility that the effects on these potentials result from a change in the afferent input (18), dorsal root action potentials were recorded extracellularly at the point where they enter the spinal cord. The findings of the experiments indicate that THC does not alter the afferent input; therefore, the results on synaptic potentials must be due to other causes. In fact, doses as

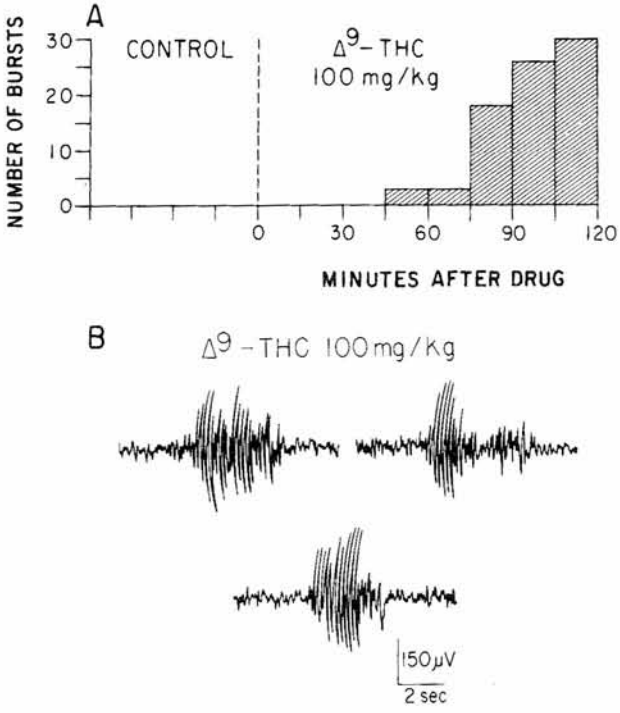

FIGURE 4. Spontaneous AD-bursts produced by THC in a cobalt epileptic rat. Bursts were recorded from the cerebral cortex at a site away from the epileptic focus. A, time course of AD-like bursts from a conscious rat Vehicle was given 15 min after the beginning of the control period; drug, at the beginning of 120-min test period. B, AD-like bursts occurring 81-90 min after THC. Similar results were observed in seven additional animals. Adapted from Ref. 5.

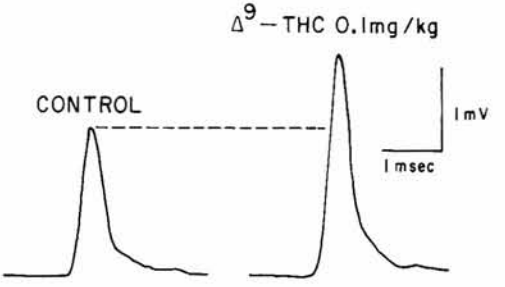

FIGURE 5. Influence of THC on the cat spinal monosynaptic reflex. The reflex was elicited by stimulating L7 dorsal roots and was recorded extracellularly from L7 ventral roots. Each response is an electronically obtained average of 16 consecutive responses recorded 5 sec apart. A, the control response 30 min after vehicle; B, response 30 min after THC. THC was studied in six cats; vehicle, five. The mean monosynaptic reflex amplitude and its range after vehicle was 98 (89-112) percent of control and after THC (0.01-0.1 mg/kg), 152 (126-185) percent of control. These values are significantly different as determined by a Mann-Whitney U statistic ($p < 0.05$) (25).

high as 10 mg/kg have no effect on the dorsal root action potential, whereas doses as low as 0.01-0.1 mg/kg elicit synaptic effects. These findings make it clear that the synapse is more sensitive to the cannabinoid than the axon.

IV. DISCUSSION

There is considerable evidence that THC can cause CNS excitation as well as the more generally recognized depression (1-3,19). The drug's excitatory character appears to manifest itself most dramatically by precipitating convulsions, especially in epileptic animals (5-7). Whether marijuana or THC will similarly affect human epilepsies must await detailed clinical assessment. The interaction with human epilepsies may, however, be very complicated because THC, like the well established antiepileptics, phenytoin and phenobarbital, elicits both anticonvulsant and convulsant effects in various laboratory tests (19); consequently, the clinical effects of the cannabinoids are likely to be as complex as are those of the antiepileptics, which exacerbate some types of epilepsies

and suppress others (20). Nevertheless, when evaluating the pharmacological properties of any cannabinoid, central excitatory effects, including the precipitation of convulsions, must be considered.

The data reported here indicate that THC produces electrophysiological manifestations of central excitation, which may contribute to its propensity to cause convulsions. First, the drug raises the firing rate of an epileptic focus resulting in the precipitation of convulsions. Secondly, it increases the duration of the AD, which represents the electrical aspect of

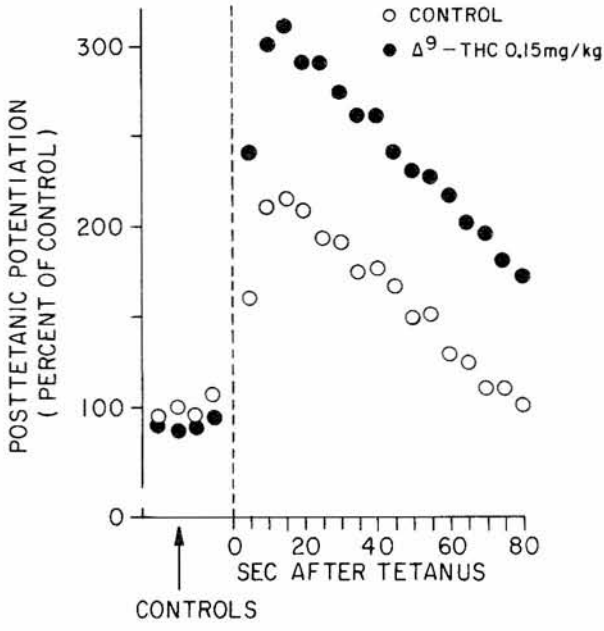

FIGURE 6. Influence of THC on posttetanic potentiation of a cat spinal monosynaptic reflex. Posttetanic potentiation is the amplitude of the posttetanic response expressed as a percentage of the pretetanic response. The responses were evoked by stimulating L7 dorsal root and were recorded from the corresponding ventral root. Similar results were observed in four additional cats.

the seizure (17), and results in a marked prolongation of the convulsion (4). This enhancement of AD duration is significant for another reason; that is, it suggests that the cannabinoid increases susceptibility to seizures (21). Thirdly, the drug promotes neurotransmission in pathways known to be important for the spread of epileptiform phenomena and the precipitation of convulsions. Lastly, the cannabinoid enhances posttetanic potentiation, which is reported to be the mechanism of action for the spread of epileptiform potentials throughout the CNS (10). Altogether, THC produces a multitude of excitatory effects on neurotransmission, and all of them may contribute to the mechanisms of the drug's convulsive actions.

Although THC clearly alters central neurotransmission, the results indicate that it does not affect axonal conduction. Our failure to observe a depression of the dorsal root action potential, however, appears to contradict the findings of Byck and Ritchie, who repored that in vitro the cannabinoid decreased the size of the compound action potential of the rabbit vagus nerve (22). The discrepancy in results may be

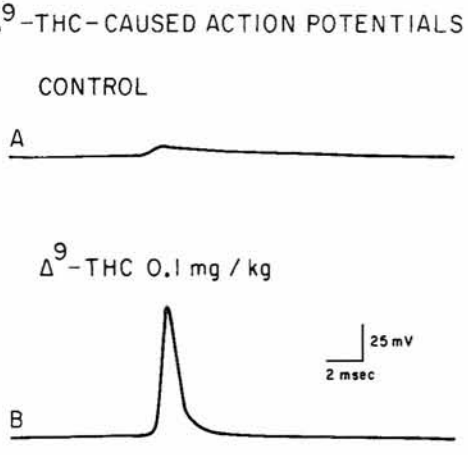

FIGURE 7. Enhancement of the production of action potentials of spinal motoneurons by THC. Intracellular recordings were made with glass microelectrodes from a cat triceps surea motoneuron. Each response is an electronically obtained average of 16 consecutive potentials obtained 5 sec apart. A, electronically averaged control EPSP 15 min after vehicle. B, electronically averaged motoneuron action potential 15 min after THC. Similar results observed in three additional experiments.

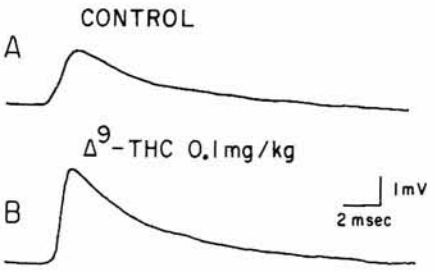

FIGURE 8. Influence of THC on EPSPs. EPSPs were recorded intracellularly from a cat triceps surea spinal motoneuron, and each response is an electronically obtained average of 16 consecutive potentials obtained 5 sec apart. A, control 15 min after vehicle; B, response 5 min after drug. In four cats, the mean EPSP amplitude and its standard deviation after THC (0.01-0.1 mg/kg) was 188 +/- 29 percent of control, which was significantly different from initial vehicle controls obtained in each experiment as determined by a t-test ($p < 0.05$) (27).

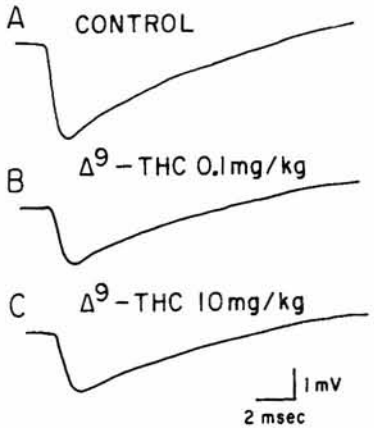

FIGURE 9. Influence of THC on IPSPs. IPSPs were recorded from a cat biceps-semitendinous spinal motoneuron, and each response is an electronically obtained average of 16 consecutive potentials obtained 5 sec apart. A, control 15 min after vehicle; B, response 15 min after drug; C, response 15 min after drug. In four cats, the mean IPSP amplitude and its standard deviation after THC (0.01-0.1 mg/kg) was 65 +/- 3.1 percent of control, which was significantly different from initial vehicle controls obtained in each experiment as determined by a t-test ($p < 0.05$) (27).

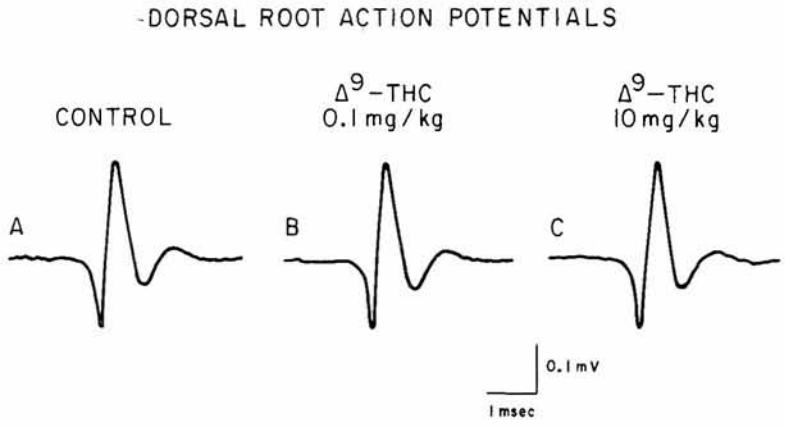

FIGURE 10. Influence of THC on afferent input. Traces were dorsal root action potentials recorded just prior to their entry into the cat spinal cord, and each response is an electronically obtained average of 16 consecutive potentials obtained 5 sec apart. A, control 15 min after vehicle; B, response 15 min after drug; C, response 15 min after drug. In four cats, the mean amplitude of the dorsal root action potential and its standard deviation after THC (0.01-0.1 mg/kg) was 101 +/- 9.0 percent of control, which was not significantly different from initial vehicle controls obtained in each experiment as determined by a t-test ($p < 0.05$) (27).

due to differences in experimental conditions: For instance, because cannabinoids have a very high partition coefficient, they will concentrate in tissues in vitro (2,9,23); consequently, the tissue concentrations in vitro may, in fact, be orders of magnitude higher than that of the bathing medium or that achievable by in vivo administration (2,9). Another possiblity is that different neurons may have different sensitivities to THC, for Brady and Carbone failed to detect any cannabinoid effect in vitro on squid-axon action potentials (24). Regardless of the reason for these discrepancies, our findings suggest that cat synapses are more sensitive to the effects of THC than are axons and, therefore, are probable sites of drug action.

The results of the spinal motoneuron experiments, in fact, point to possiible synaptic mechanisms for the cannabinoid's convulsive action; that is, the drug elicits excitation by increasing the amplitude of the EPSP and partially reducing the amplitude of the IPSP. The limited effect on IPSPs may explain why the cannabinoid is not a strychnine-like convul-

sant and why its convulsive responses only occur in subjects with a propensity towards convulsions, such as the epileptic rats described here, epileptic beagles (7) and a special strain of rabbits (8). Nevertheless, if the spinal motoneuron findings are applicable to higher centers, they provide feasible synaptic mechanisms for THC's convulsive properties.

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