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Cannabidiol-Caused Depression of Spinal Motoneuron Responses in Cats

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TURKANIS, S. A. AND R. KARLER. *Cannabidiol-caused depression of spinal motoneuron responses in cats.* PHARMACOL BIOCHEM BEHAV **25**(1) 89–94, 1986.—Intracellular recording techniques were used on spinal motoneurons in the cat in order to define the synaptic pharmacology of cannabidiol (CBD). The cannabinoid produces only depression of electrophysiological responses of the motoneurons: For instance, the drug decreases the amplitude of excitatory postsynaptic potentials (EPSPs); this reduction does not appear to be the result of a change in the afferent input. In addition, CBD raises the firing threshold and decreases the amplitude of motoneuron action potentials; the effects on action potentials are related to changes in postsynaptic membrane conductances, probably involving at least sodium conductance. The spinal motoneuron effects provide potential electrophysiological mechanisms for CBD's central depressant actions.

Cannabidiol Mechanisms of action Synaptic effects Membrane effects Spinal motoneuron Electrophysiological properties

CANNABIDIOL (CBD), like delta-9-tetrahydrocannabinol (THC), is a major, naturally occurring marijuana constituent which exhibits some depressant actions characteristic of marijuana [8]: For instance, CBD, as well as THC, elicits anticonvulsant effects, sedation and motor toxicity [8]; CBD also increases brain concentrations of THC by interfering with its metabolism [7]. Both the metabolic and pharmacodynamic effects of CBD may account for the observations that the drug enhances depressant actions of THC [9]. The primary reason for studying CBD is that by virtue of its depressant effects the drug may contribute to the toxicity of marijuana. A secondary consideration is that CBD displays therapeutic potential as an antiepileptic because in humans it effectively suppresses complex-partial seizures [2]; in fact, the cannabinoid may offer therapeutic advantages over phenytoin and carbemazepine, which are of limited efficacy in the treatment of these seizures [12]. Because of CBD's toxicological and therapeutic potential, the present electrophysiological investigation was carried out to define its synaptic pharmacology; to this end, the effects of CBD were studied on individual spinal motoneurons in the cat. The goal of the work was to investigate possible mechanisms of the cannabinoid's central depressant actions, especially those that contribute to its potential toxic and therapeutic effects.

METHOD

Experimental Preparation and Procedures

The methods for the study of spinal pathways in cats with their spinal cords severed and their brains destroyed ischemically have been described previously [4, 16–18]. In brief, the results were obtained from 30 different adult female cats weighing 2.7–3.3 kg; in each of 28 cats, only one motoneuron was evaluated pharmacologically; therefore, each motoneuron served as its own control. In two other cats, resting membrane potentials were measured in six motoneurons following vehicle and in six different motoneurons following 5 mg/kg IV of CBD. The number of cats used in the different types of experiments were as follows: eight for the CBD-EPSP experiments (4 for each dosage range), ten for the CBD-antidromic action potential experiments (four for the low dosage range; six for the high dosage range), six for the THC-antidromic action potential experiments (high dosage range only), two for CBD-resting membrane potential population experiments, four for the CBD-IPSP experiments (high dosage range only). The effects of CBD on the EPSP and dorsal root action potentials were studied in the same eight cats (four for each dosage range). The cats were anesthetized with a single dose of sodium pentobarbital (40 mg/kg IP); pentobarbital was selected because the basic physiology of the cat spinal cord has been mainly defined in pentobarbital-treated animals [1, 4, 11]. For purposes of comparison, four additional cats were initially anesthetized with ether; the data obtained from these animals were comparable to those from cats given pentobarbital; the similarity in findings was important because the results with ether serve to support the validity of the data obtained in the presence of pentobarbital.

After an animal was anesthetized, a tracheal cannula was inserted and standard procedures were carried out to produce ischemic brain destruction; that is, throughout the remainder of the experiment the carotid arteries were ligated, and the vertebral arteries were clamped. The spinal cord was then sectioned at the atlanto-occipital junction, and artificial respiration was initiated. During each experiment, the ventilation parameters were selected so that arterial blood pH, bicarbonate concentration, pCO_2 and pO_2 values were similar to those previously reported for the cat [3, 5, 6].

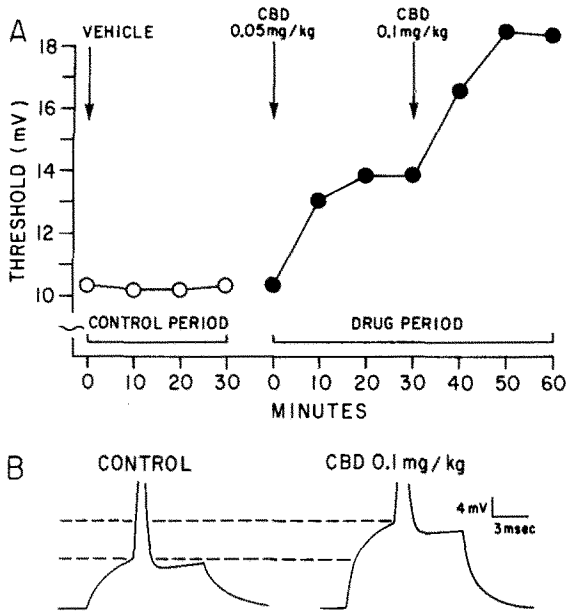

FIG. 1. CBD-caused increase in threshold of motoneuron action potential. The results were obtained from a triceps surae motoneuron. A. Time course of drug effect; the arrows indicate the time of vehicle and drug administration. Open circles represent control values; filled circles, values after drug. B. Responses, 20 min after vehicle and 20 min after drug (0.1 mg/kg). The action potentials were evoked and recorded with a single intracellular microelectrode by means of an active bridge circuit. The firing thresholds are indicated by the dashed lines.

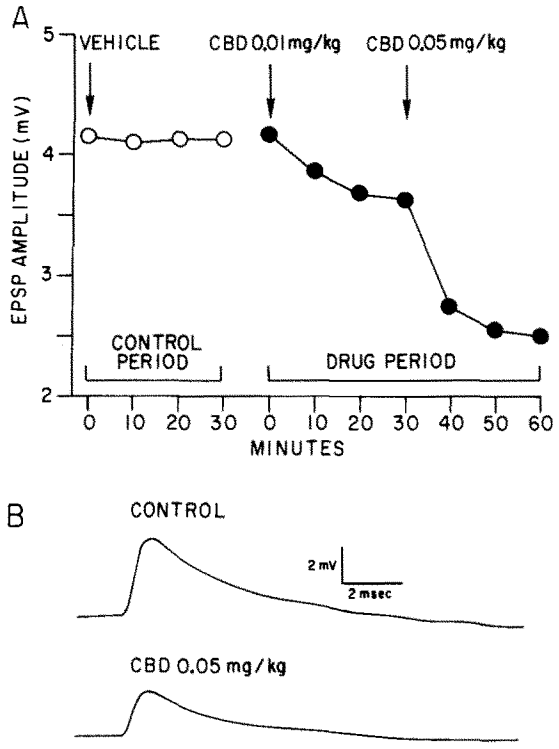

FIG. 2. CBD-caused depression of EPSP amplitude. The results were obtained from a triceps surae motoneuron. A. Time course of drug effect, the arrows indicate the time of vehicle and drug administration. Open circles represent control values; filled circles, values after drug. B. Each response is the electronic average of 16 consecutive potentials. Responses, 30 min after vehicle and 30 min after drug (0.05 mg/kg).

These measurements were carried out periodically during each experiment with a Radiometer Acid-Base Laboratory (ABL 1); and the mean values and their standard deviations obtained from all the cats were: pH, 7.44±0.07; bicarbonate concentration, 19.8±1.3 mM/l; pCO_2, 31.0±2.2 mmHg; pO_2, 91.0±9.0 mmHg; in addition, end-tidal CO_2 concentration was continuously monitored with an Infrared Gas Analyzer (Model 703).

Body temperature was maintained at about 37–38°C, and blood pressure ranged between 70–100 mmHg. CBD and THC did not cause changes in blood pressure. Animals were treated with gallamine triethiodide (6 mg/kg, IV) to produce neuromuscular paralysis, and supplemental doses were administered as needed.

Experimental Design and Data Processing

Peripheral muscle nerves (the triceps surae and posterior biceps-semitendinosus) in the hindlimb of the cat were electrically stimulated every 5 sec, and the resulting EPSPs and action potentials were recorded intracellularly from single spinal motoneurons [4]; inhibitory postsynaptic potentials (IPSPs) were elicited by stimulating the quadraceps muscle nerves. Compound action potentials were recorded extracellularly from the dorsal roots near the point where they enter the spinal cord in order to quantify the afferent input.

In general, conventional electrophysiological methods for intracellular recording from spinal motoneurons were used; that is, 10–15 megohm glass microelectrodes, filled with 3-M potassium acetate solution were employed to measure EPSPs, IPSPs, motoneuron action potentials and membrane resistance [4]. A single intracellular microelectrode and an active bridge circuit were used to evoke and record potentials from the soma. The bridge circuit was balanced with the aid of square wave prior to penetrating a motoneuron with the microelectrode and was still balanced upon withdrawal of the microelectrode from the neuron at the end of each experiment. Furthermore, electrode resistance did not change during the experiments. The threshold was defined as the magnitude of the electrical depolarization required to produce an action potential. In general, the various electrophysiological responses were averaged by the use of a signal processor (Nicolet 1174); then, electronically obtained averages were drawn by an X-Y plotter (Honeywell 530).

As illustrated in Figs. 1–4, a dose of vehicle was given and at least 30 min of relatively stable and reproducible electrophysiological responses were obtained, then a dose of drug was administered every 30 min. In each experiment, intracellular recording was maintained for at least 90 min, that is, a 30-min control period and a 60-min drug period (see Figs. 1–4). The maximum changes after drug were expressed as a percentage of the initial vehicle control values obtained in each experiment. The amplitude of the antidromic action potential of the motoneuron was measured periodically dur-

TABLE 1

INFLUENCES OF CANNABIDIOL ON THE AFFERENT INPUT AND SPINAL MOTONEURON PARAMETERS

Parameter	Dosage Ranges 0.01–0.1 mg/kg	1–5 mg/kg
Firing Threshold for Motoneuron Action Potential	173 ± 18*	†
EPSP Amplitude	59 ± 6.1*	53 ± 5.8*
Dorsal Root Action Potential Amplitude‡	101 ± 9.0	99 ± 7.2
Motoneuron Action Potential Amplitude	98 ± 7.7	48 ± 10*

Data are mean values and their standard deviations of the maximum effects caused by CBD and are expressed as percent of initial control obtained in each experiment; each mean was calculated from the results obtained from four motoneurons or four dorsal roots. Because CBD (0.01–0.1 mg/kg) did not affect the antidromic action potential in the four initial motoneuron experiments reported above, this parameter was used as a measure of the physiological status of the cells in subsequent low dose studies (also see the Method and Results sections). The data summarized in Table 1 were obtained from 20 cats; only one motoneuron was studied in each cat. In those cats in which the EPSPs were recorded, the dorsal root action potentials were also measured. Therefore, 12 cats were used in low dosage range (0.01–0.1 mg/kg) studies, and eight for the high dosage range (1–5 mg/kg) studies. The mean control values and their standard deviations were: amplitude of antidromic motoneuron action potential, 81 ± 2.1 mV; excitatory postsynaptic potential (EPSP) amplitude, 5.1 ± 0.6 mV; dorsal root action potential amplitude, 0.35 ± 0.1 mV; threshold for motoneuron action potential, 11.0 ± 0.9 mV.

*Significantly different from initial vehicle controls obtained in each experiment, as determined by a t-test ($p<0.05$) [13].

†The effects of high doses on threshold were not reported because it was not possible to evoke an action potential consistently.

‡The duration and conduction velocity of the compound action potential recorded from the dorsal roots were also unaffected by CBD.

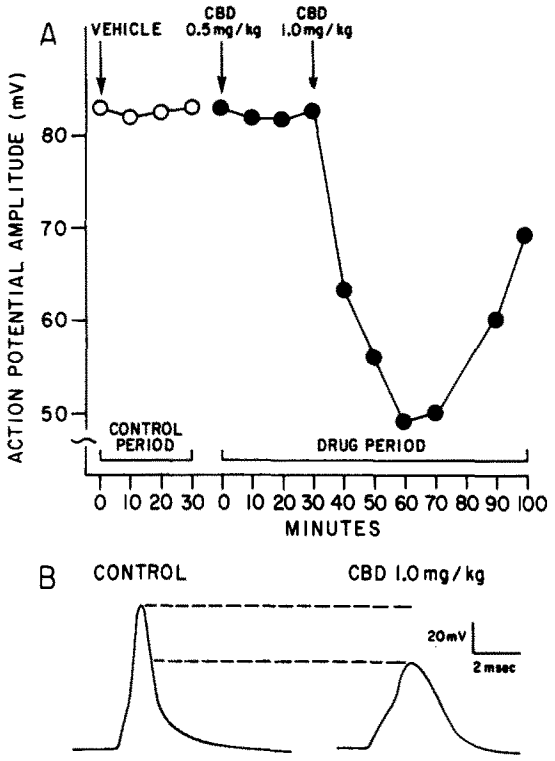

FIG. 3. CBD-caused depression of amplitude of the motoneuron action potential. A. Time course of the drug effect; the arrows indicate the time of vehicle and drug administration. Open circles represent control values; filled circles, values after drug. B. Responses, 30 min after vehicle and 30 min after drug (1 mg/kg). The responses were evoked antidromically. The means and standard deviations of the motoneuron membrane resistance were 1.3±0.3 megohms during the control period and 1.3±0.2 megohms during the drug period; the resting membrane potential at the end of the drug period was 74 mV.

ing experiments and was used as an indicator of the physiological status of the motoneuron. To be included in the study, a potential had to be at least 75 mV; if the action potential amplitude decreased more than 5 mV, the data were not used. An additional indicator of the physiological status of the neurons was that at the end of the experiments, the motoneuron action potentials displayed an overshoot of 10 mV or greater. The membrane resistance of the motoneuron was also determined, and it also served as a measure of the condition of the neuron. These criteria for assessing the physiological status of the motoneurons were developed by M. Kuno (personal communication).

Drug Preparation

Cannabinoids were dispersed by ultrasound in isotonic sodium-chloride solution with Tween 80 [14]; the drug and vehicle preparations contained a final concentration of 0.25% Tween 80. Cannabinoid and their vehicle preparations were administered slowly via a cannula in the cephalic vein; as previously noted, the vehicle exerted no measurable effects [16,17]. In general, drugs were given in progressively increasing steps with 30-min intervals between doses. The interval between doses was selected because, in general, cannabinoid effects reached their maximum within 30 min.

RESULTS

CBD depresses the electrophysiological parameters of spinal motoneurons: For instance, the cannabinoid (0.01–0.1 mg/kg) increases the threshold of motoneuron action potentials (Fig. 1 and Table 1); the effect appears to be dose related. An additional depressant response that occurs within the same dosage range is a decrease in EPSP amplitudes (Fig. 2 and Table 1); again, the effect appears to be dose related. Higher doses (1–5 mg/kg) cause little additional reduction in the EPSP; therefore, CBD appears to have a limited efficacy for depressing the synaptic potential (Table 1). The observed decrease in the EPSP is unlikely to be the result of a change in the afferent input because the drug exerts no effect on the compound action potential recorded from the dorsal roots (Table 1).

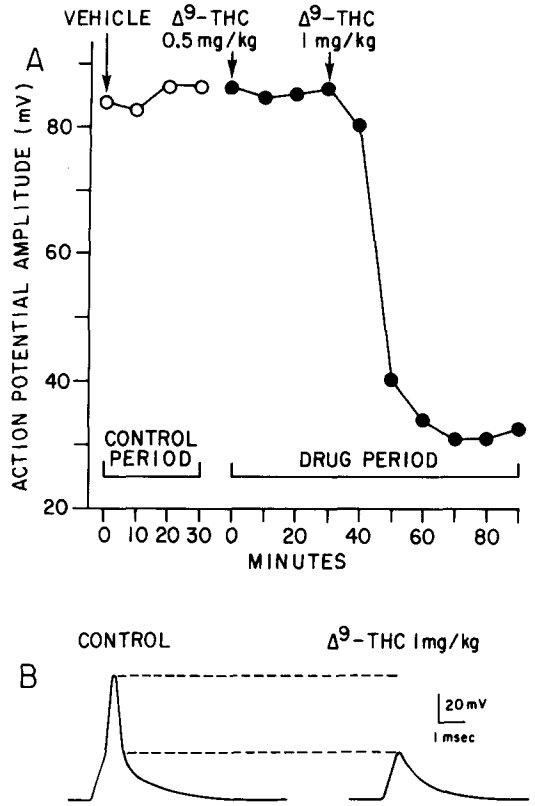

FIG. 4. THC-caused depression of amplitude of the motoneuron action potential. A. Time course of the drug effect; the arrows indicate the time of vehicle and drug administration. Open circles represent control values; filled circles, values after drug. B. Responses, 30 min after vehicle and 30 min after drug (1 mg/kg). The responses were evoked antidromically. The means and standard deviations of the motoneuron membrane resistance were 1.1±0.2 megohms during the control period and 2.1±0.5 megohms during the drug period; THC-caused increases in motoneuron membrane resistance have been previously reported [16]. The resting membrane potential at the end of the drug period was 68 mV.

In the above threshold and EPSP studies involving the low dosage range (0.01–0.1 mg/kg), the amplitude of the antidromic action potential and the membrane resistance were also measured at least every 5 min in each motoneuron in order to assess the physiological condition of the cells. The means and standard deviations of the amplitudes of the antidromic action potentials from eight motoneurons were 81±3.5 mV during the control periods and 82±3.4 mV during the drug periods. The means and standard deviations of the membrane resistance in the same eight motoneurons were 1.2±0.2 megohms during the control period and 1.3±0.2 megohms during the drug period. At the end of each threshold and EPSP experiment, the microelectrode was withdrawn from the motoneuron in order to determine the resting membrane potential and the action potential overshoot; in the eight cells, the means and standard deviations were 71±3.1 mV for the resting membrane potentials and 11±0.8 mV for the action potential overshoot. The values for the amplitude and overshoot of the action potential, membrane resistance, and resting membrane potential are comparable to those previously published [4,10]. Such data support several conclusions: First, the cells were in good physiological condition throughout the control and drug periods. Secondly, the CBD effects, such as the decrease in the EPSP, were not due to a drug-caused change in membrane resistance. Last, because CBD does not affect the amplitude of the antidromic action potential, changes in resting membrane potential do not appear to contribute to the responses to CBD.

TABLE 2

A COMPARISON OF THE EFFECTS OF CBD AND THC ON AFFERENT INPUT AND SPINAL MOTONEURON PARAMETERS

Parameter	CBD*	THC*
Firing Threshold for Motoneuron Action Potential	increase	increase
Motoneuron Action Potential Amplitude†	decrease	decrease
EPSP Amplitude	decrease	increase
Membrane Resistance	no effect	increase
IPSP Amplitude	no effect	decrease
Dorsal Root Action Potential	no effect	no effect

*Effects occur within a 0.01–0.1 mg/kg IV dosage range, except that the depression of the antidromic action potential amplitude requires 1–5 mg/kg. The THC findings, except for effects on the amplitude of the action potential, have been previously published [16].
†Antidromic action potentials.

The effects of high doses of CBD, that is 1–5 mg/kg, were investigated on the amplitude of the antidromic action potential in four motoneurons; under these conditions, CBD markedly reduced the action potential (Table 1 and Fig. 3). In general, the antidromic action potential amplitude tends to return towards control values within 30–40 min (Fig. 3). In the same four motoneurons, the means and standard deviations of the membrane resistance were 1.3±0.1 megohms during the control periods and 1.2±0.1 megohms during the drug periods, and the mean and standard deviation of the resting membrane potential of motoneurons at the end of the drug periods were 68±3.9 mV. Again, the motoneurons appear to be in good physiological condition. In two additional motoneurons, the microelectrode was withdrawn from the soma at the drug's peak-effect time for the action potential depression in order to measure their resting membrane potentials, which were 74 and 71 mV. In addition, the resting membrane potentials were also determined following vehicle and CBD treatments in two other cats; the means and standard deviations were 70±3.3 mV in six motoneurons 20–40 min after vehicle and 71±3.6 mV in six different motoneurons 20–40 min after 5 mg/kg of CBD. These data support the conclusion that CBD does not alter the resting potential at the time that the drug produces its peak depression of the action potential; consequently, changes in the resting membrane potential appear to make little or no contribution to the decrease in the action potential amplitude.

For purposes of comparison, similar THC experiments were carried out with four motoneurons; the results of the THC experiments were essentially indistinguishable from those with CBD: specifically, 1–5 mg/kg of THC was required to reduce the amplitude of the action potentials; that is, the mean and standard deviation were 36±5.2 percent of control values (Fig. 4). In the same four motoneurons, the means and standard deviations of the membrane resistance

were 1.3±0.2 megohms in the control periods and 2.2±0.4 megohms during the drug periods; the THC-caused increase in motoneuron membrane resistance has been previously reported [16]. The mean and standard deviation of the resting potential in the same motoneurons at the end of the drug periods were 69±1.7 mV. Such data also indicate that the motoneurons are in good physiological condition. In two additional motoneurons, the resting membrane potentials measured at THC's peak depressant effect were 70 and 72 mV; again, changes in the resting potential do not appear to contribute to the cannabinoid-caused decrease in the action potential amplitude.

Because neither THC or CBD affect the compound action potential recorded from the dorsal root (Tables 1 and 2), action potentials recorded from the soma appear to be more sensitive to the effects of these drugs than those of axons. In four motoneurons, CBD had no effect on the IPSP; that is, the means and standard deviations of the amplitudes of the IPSP were 3.0±0.7 mV after vehicle and 2.9±0.9 after 1–5 mg/kg of CBD.

DISCUSSION

The results of the present study indicate that CBD produced depression of electrophysiological responses of spinal motoneurons; specifically, it reduced the amplitudes of EPSPs and motoneuron action potentials and it raised the firing threshold for motoneuron action potentials (Tables 1 and 2). Findings of comparable studies indicate that THC, like CBD, raised the firing threshold and reduced the amplitude of motoneuron action potentials (Fig. 4 and Table 2) [16]. In addition, THC, unlike CBD, elicited excitation as well as depression; in this respect, THC caused excitation by increasing the EPSP and decreasing the IPSP (Table 2) [16]. The data reported here, however, clearly demonstrate that CBD shares some of THC's electrophysiological depressant properties on individual spinal motoneurons. The similarity in depressant actions of the two cannabinoids on synapses provides one feasible mechanism by which CBD may enhance some of the depressant effects of THC [9].

Another point is that responses to CBD observed at individual neurons resemble those in conscious animals and those from pools of neurons. In conscious animals, CBD produces depressant responses, such as anticonvulsant effects and motor toxicity [8,14], and in pools of neurons, CBD also causes depression; that is, a reduction of cortical evoked responses, limbic afterdischarges and spinal monosynaptic reflexes [15]. The generality of the drug effects at each level of organization studied suggests that effects on single neurons may contribute to the mechanisms of action of the cannabinoid's depressant properties. For example, if the effects on single spinal motoneurons are applicable to higher centers, then they may account for the cannabinoid's anticonvulsant activity. In this respect, CBD raises thresholds for electrically induced minimal convulsions in conscious animals [8,14], and the drug raises the threshold for electrically induced afterdischarges (seizure discharges) in pools of limbic neurons [15]. Both effects reflect the drug's anticonvulsant activity, and both effects may be the direct result of an increased firing threshold in individual neurons. Because THC raises the same three thresholds as does CBD, identical conclusions can be drawn for THC's anticonvulsant mechanisms of action [8,15].

From the data reported above, it is not possible to ascertain if the site of action of the CBD-induced decrease in EPSP amplitude is pre-or postsynaptic. In contrast, the CBD-caused increase in firing threshold and reduction in amplitude of the motoneuron action potential are related to changes in postsynaptic membrane conductances, probably at least involving sodium conductance. Whatever the precise cellular sites and mechanisms of action, if these drug responses occur in higher centers, they provide feasible electrophysiological mechanisms for CBD's central depressant actions, which may contribute to its potential toxic and therapeutic effects.

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