DELTA-9-TETRAHYDROCANNABINOL: SITE OF ACTION FOR AUTONOMIC EFFECTS*

Michael J. Hosko
William T. Schmeling
Harold F. Hardman

Department of Pharmacology and Toxicology
Medical College of Wisconsin
Milwaukee, Wisconsin

I. INTRODUCTION

Numerous studies have demonstrated that the cannabinoids are potent agents capable of producing a spectrum of pharmacological effects. These actions range from subtle psychoactive effects that almost certainly are mediated at forebrain and diencephalic loci, to autonomic effects that are manifested by alterations in respiration, blood pressure, temperature regulation and heart rate.

Studies from this as well as from other laboratories demonstrate that delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of marijuana, has pronounced ability to induce hypotension and bradycardia in experimental animals. The ability of THC to induce hypotension is not compromised by transection of the neuraxis at the midcollicular level but is abolished by high cervical section. Figures 1 and 2 demonstrate the effects of systemically administered THC on blood pressure and temperature in cats with midcollicular and high cervical transections. The neuraxis was transected under halothane anesthesia. In the case of the midcollicular transections, the forebrain was destroyed by coagulation before discontinuing anesthesia. In the C-1 cord transected animals, a large needle was passed axially along the rostral reticular formation and a coagulating current was

*Supported by NIDA grant DA-00124.

applied to destroy the brainstem before discontinuing anesthesia. The animals were maintained on artificial respiration.

Intravenous administration of THC to midcollicular transected animals produced significant decreases in blood pressure (Figure 1) and heart rate when compared to vehicle controls. Within 30 minutes after THC administration, body temperature was significantly depressed when compared to controls (Figure 2). The hypotension and bradycardia elicited by THC administration in these unanesthetized cats with midcollicular transections agree well with previous reports using anesthetized cats with similar transections, and is comparable to the responses obtained in intact anesthetized cats (1). Administration of the second dose (4.0 mg/kg) of THC, produced

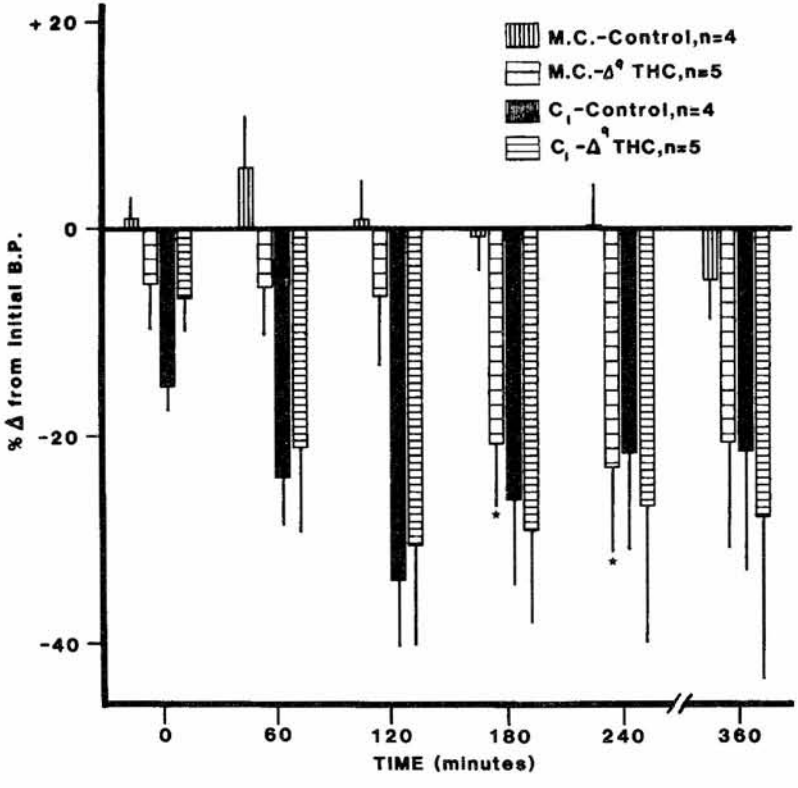


FIGURE 1. Effect of THC on blood pressure in midcollicular (MC) and spinal (C-1) transected cats. Control animals received vehicle in appropriate volume at 60, 120, and 240 minutes. The THC animals received vehicle at 60 minutes, 2 mg/kg THC (i.v.) at 120 minutes and 4 mg/kg THC (i.v.) at 240 minutes.

minimal additional effects. The somatic effects produced by the cannabinoids characteristically exhibit tachphylaxis in experimental animals. Vehicle produced no significant effects on body temperature, heart rate, or blood pressure.

The C-1 transected animals were used to establish the rate of body temperature loss in cats incapable of significant centrally mediated thermoregulation. Figure 2 demonstrates that the rate of temperature loss in C-1 transected animals receiving THC was statistically indistinguishable from C-1 transected animals receiving vehicle alone. These data indicate that in C-1 transected preparations, THC produced few if any peripheral effects that contribute to body temperature loss. The cardiovascular effects of THC were also absent in

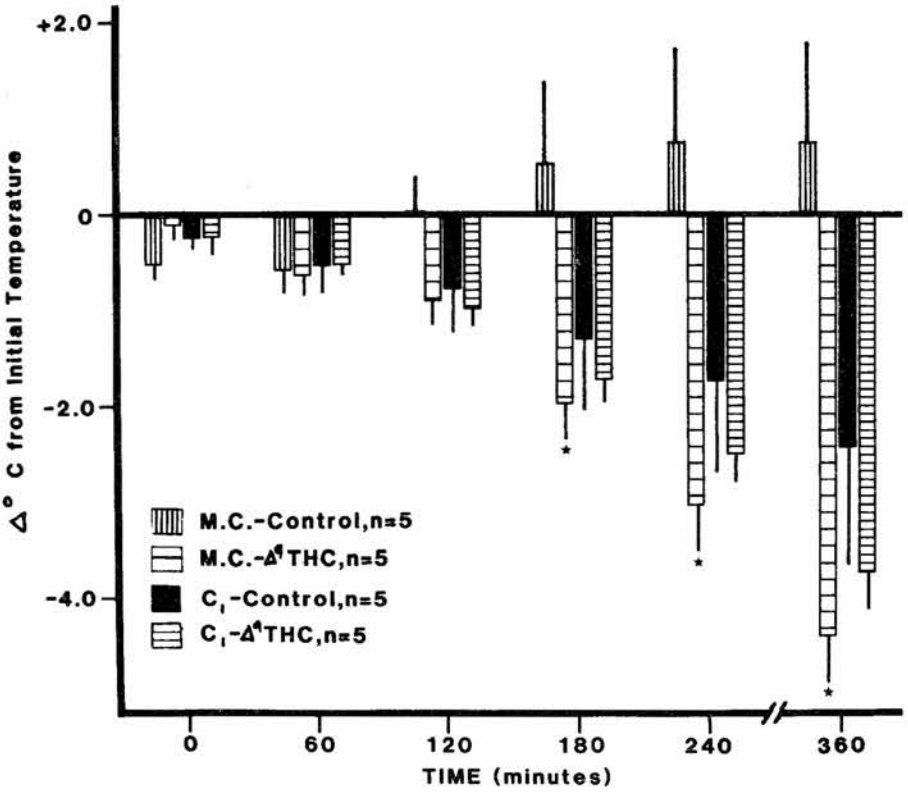
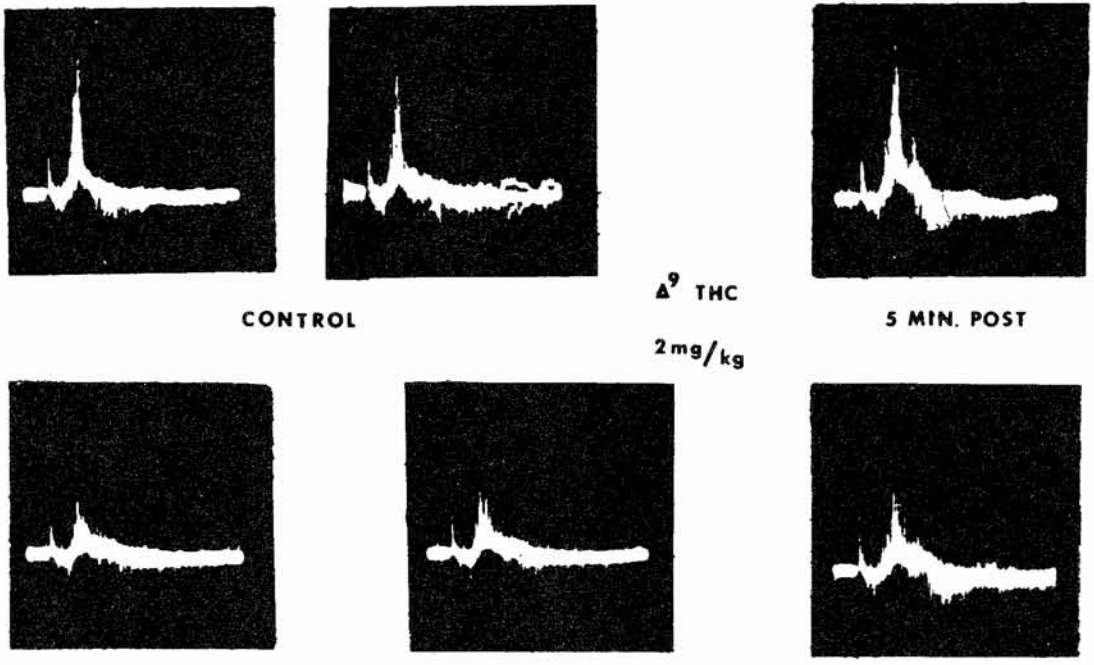


FIGURE 2. Effect of THC on body temperature in midcollicular (MC) and spinal (C-1) transected animals. Control cats received vehicle in appropriate volume at 60, 120 and 240 minutes. The THC animals received vehicle at 60 minutes, 2 mg/kg THC at 120 minutes ad 4 mg/kg THC at 240 minutes.

I.L.C. RESPONSES EVOKED FROM HYPOTHALAMUS



C-1 transected animals (Figure 1). Figure 2 further demonstrates that administration of THC to midcollicular preparations resulted in a rate of body temperature loss that closely approximated the rate seen in C-1 transected (control) animals.

In summary, the hypothermic response to THC, however mediated, was largely absent after low neuraxis transection at C-1, but was essentially unaffected by higher midcollicular transections which spared neuronal pools that subserve the autonomic nervous system. The hypothermic response exhibited by the midcollicular transected animals was essentially the same as that observed in intact animals (2).

In order to test the hypothesis that THC may exert some of its cardiovascular effects by disrupting sympathetic outflow, we have monitored evoked activity in the intermediolateral cell column (ILC) of the thoracic cord before and after THC administration (3). Hypothalamic cardiovascular pressor centers project to the ILC by monosynaptic as well as by polysynaptic pathways via the rhombencephalon.

II. RESULTS AND DISCUSSION

Male and female cats were anesthetized with chloralose-urethane IP. The femoral artery and vein were cannulated for recording BP and for administration of drugs. After appropriate surgical exposures, a coaxial stimulating electrode was positioned in the hypothalamus to elicit pressor responses. A tungsten recording microelectrode was positioned in ILC between T-1 and T-5. The hypothalamic electrode was used to deliver single square wave pulses (0.3 msec, 0.5-3.0 V) at 0.5 Hz. Figure 3 illustrates typical potentials evoked in ILC by stimulation of hypothalamic pressor sites. Each trace represents 10 superimposed responses to stimuli delivered at 2 second intervals. Five minutes after THC administration the evoked activity in the ILC is augmented. The augmented

FIGURE 3. Effects of THC on intermediolateral cell column field potentials evoked by stimulation of a hypothalamic pressor site in the cat. Each trace represents 10 superimposed potentials. Controls were taken 15 to 30 minutes after vehicle was administered. Panel A, 5 minutes after THC (2 mg/kg i.v.) increased amplitude coincided with a transient blood pressure increase. Panels B, C, D = 15, 30 and 60 minutes post THC. Blood pressure was reduced during B and C and was beginning recovery at D.

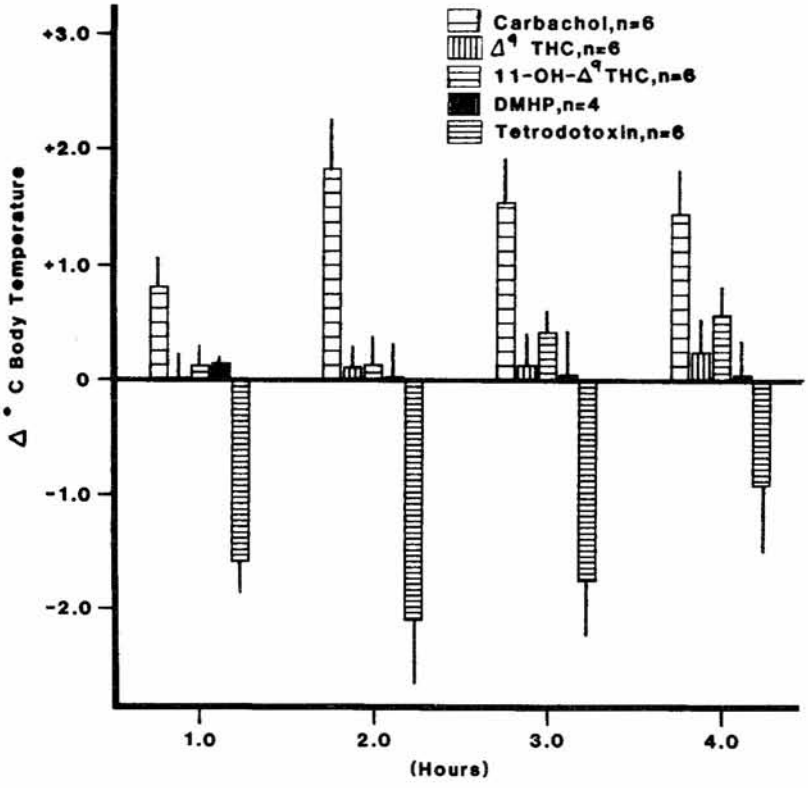


FIGURE 4. Lack of effect of microinjected cannabinoids on telemetered deep body temperature after administration to proven chemosensitive sites of the preoptic hypothalamus in chronic, unrestrained cats. Both carbachol and tetrodotoxin induced significant changes in body temperature. The effects of the cannabinoids were within the temperature variation monitored during a 2 hour vehicle control period. Drugs were administered at four to seven day intervals in a Latin square design. Carbachol, 5.5 mcg; THC, 100 mcg; 11-hydroxy-THC, 100 mcg; dimethylheptylpyran, 100 mcg; tetrodotoxin 75 ng.

response coincided with a slight, transient increase in blood pressure which sometimes occurs after IV THC administration. At 15 through 30 minutes post THC, the evoked responses in ILC are depressed. This time span corresponds to the period of peak blood pressure depression. By 60 minutes the evoked response is returning toward control levels. This coincides with the return of blood pressure toward control levels.

These data suggest that THC alters central processing of cardiovascular afferent input as well as sympathetic outflow

to the cardiovascular system.

The cardiovascular response to cannabinoid administration is severely compromised by high spinal section (C-1), but not by transection at midcollicular levels. Attenuation of the pressor response to common carotid occlusion and the depressor response to carotid sinus nerve stimulation has been demonstrated after administration of THC (4). These findings support the conclusion that the primary site responsible for the cardiovascular actions of the cannabinoids is between the mesencephalon and C-1.

In a previous study from this laboratory (5), we demonstrated that in the rat, the preoptic region of the anterior hypothalamus was not the primary site of action for THC hypothermia. Studies in cats with brainstem transections at various levels along the neuraxis indicate that the primary site

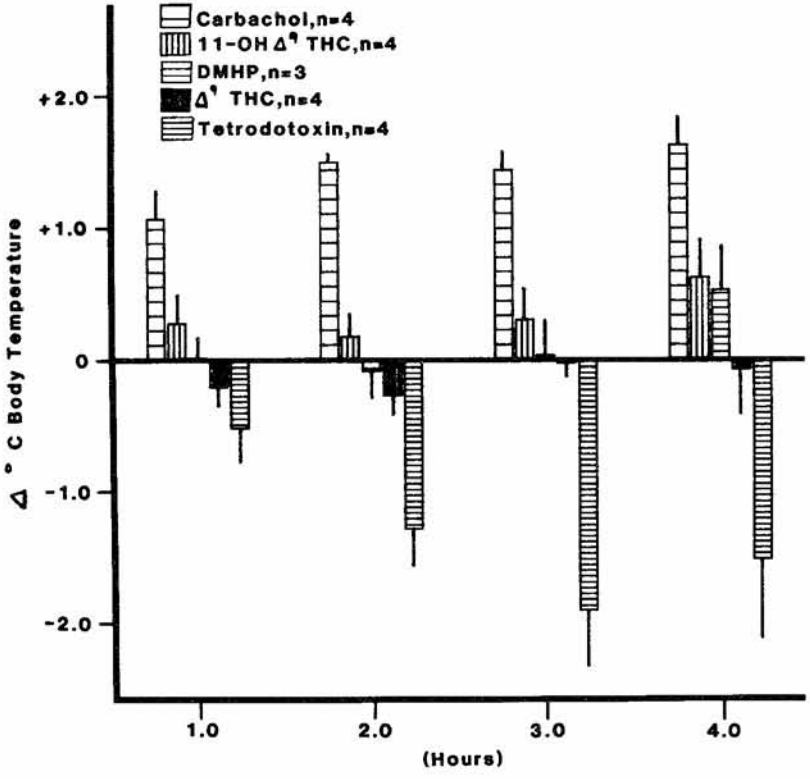


FIGURE 5. Lack of effect of microinjected cannabinoids on telemetered deep body temperature after administration to proven chemosensitive sites in the posterior hypothalamus in chronic, unrestrained cats. See Figure 4 for details.

of action is in the caudal brainstem. To further challenge this hypothesis we applied cannabinoids directly to proven hypothalamic chemosensitive thermoregulatory sites in cats with implanted microinjection guides. In order to allow undisturbed observation of behavioral response to the microinjection, temperature telemetry units were implanted into the peritoneal cavity at the same time the guide tubes were implanted.

Following surgery, the animals received 100,000 units of penicillin for three consecutive days. A minimum of ten days was allowed for recovery.

Each animal received the following drugs: 11-Hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC), 100 mcg (5.0 mcl); delta-9-tetrahydrocannabinol (THC), 100 mcg (8.9 mcl); carbamylcholine (Carbachol), 5.5 mcg (3.0 mcl); tetrodotoxin, 75 ng (1.5 mcl); dimethylheptylpyran (DMHP), 100 mcg (8.0 mcl). The drugs were microinjected into the target sites at five- to seven-day intervals using a Latin square design.

Figures 4 and 5 demonstrate that while carbachol and tetrodotoxin evoked statistically significant alterations in body temperature, intracerebral microinjection of THC, 11-OH-THC or DMHP at doses of 100 mcg produced little or no effect. In order to control for the various vehicles utilized, sterile water, saline and one and two times the normal cannabinoid vehicle concentration (all 8.0 mcl volume) were microinjected into documented chemosensitive sites. No significant alteration in body temperature was observed. To insure that the lack of response to THC microinjection was not due to an insufficient dose, doses of THC as high as 400 mcg (8.0 mcl) were made into proven chemosensitive sites in four cats before the animals were sacrificed. Even at these doses, no significant temperature effects were elicited.

It should be noted that tetrodotoxin and carbachol were included in the cannabinoid microinjection series in a sequence determined by the Latin square design. In every instance, the response elicited by the second dose virtually replicated the thermal and behavioral response elicited by the test dose that established chemosensitivity for the site. This observation indicated that the initial administration of carbachol and tetrodotoxin or, subsequently, the rather large volume (8 mcl) used to administer the cannabinoids did not functionally damage the target site. The differential behavioral effects elicited by the various test agents administered into different sites further suggest that there was minimal, if any, diffusion of the drugs into the ventricles.

At the end of the microinjection sequence, parenteral administration of THC, 11-OH-THC and DMHP (1 or 2 mg/kg) produced hypothermia in every animal, demonstrating that the implanted animals were capable of responding to the test

agents. The magnitude of the hypothermic response to parenterally administered agents was consistent with previously published results. The animals were able to respond to external stimuli, however, they made no attempt to conserve body temperature, i.e., there was no evidence of shivering, piloerection or curling or huddling of the body. Deep core temperature recorded 24 hours after intraperitoneal or intravenous injection was always at or near control levels. In general, administration of the cannabinoids by the intravenous route produced a greater hypothermic response than that produced by the intraperitoneal route (6).

We have shown acute lateral intraventricular injection of cannabinoids to induce significant hypothermia in a number of different species. Figure 6 compares the hypothermic action

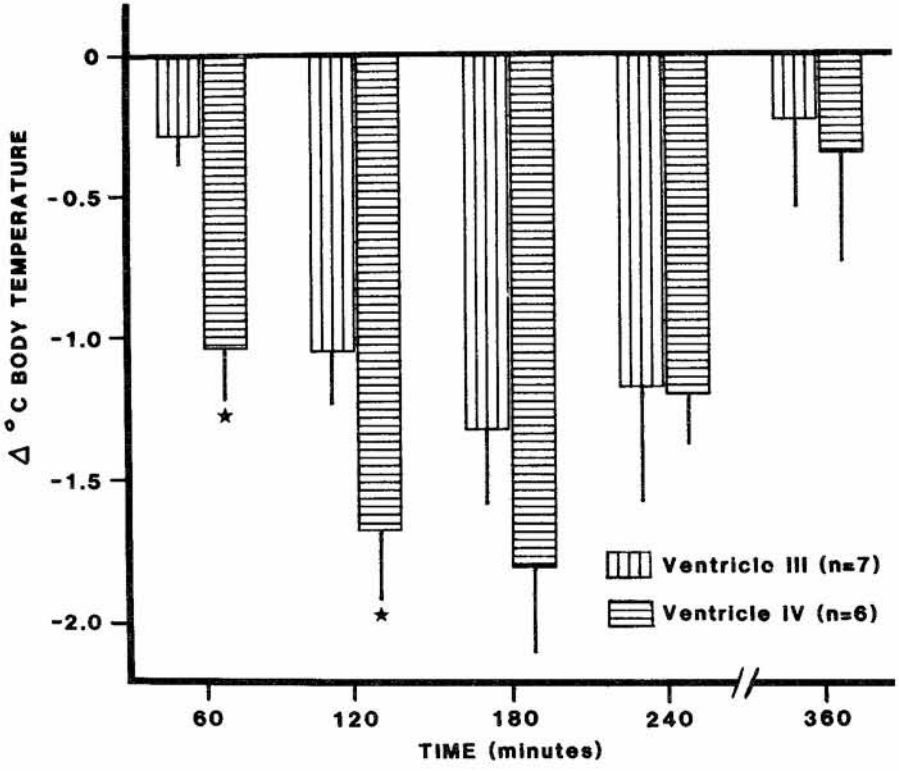


FIGURE 6. Hypothermia induced by THC administered to Ventricle III and IV in chronic unrestrained cats. Temperature was monitored by telemetry. Vehicle produced no significant hypothermia. *Significantly different from Ventricle III administration.

of THC after administration into the third and fourth ventricle in chronically implanted unanesthetized cats. In order to avoid handling the animals, temperature was recorded by telemetry.

Microinjection of 100 mcg THC (in 8.0 mcl) into ventricle III elicited minimal hypothermic effects. Microinjections of 500 mcg of THC (in 40.0 mcl) produced a significant hypothermic response. Onset of temperature depression was slow with little effect during the first hour post-injection. Peak hypothermia, 1.0-2.5°C, was reached 2-4 hours after microinjection. Behavioral manifestations included bilateral nictitating membrane relaxation, mild ataxia, and tremor, slight decrease in response to auditory or tactile stimuli, and some autonomic effects including defecation, urination, and emesis.

Microinjection of THC (500 mcg in 40.0 mcl) into ventricle IV produced significant hypothermic responses. Temperature decrease began within 15 min of administration and reached peak levels at 1.5-3.0°C within 1-2 hours. After injection, cats exhibited bilateral pupillary dilation and nictitating membrane relaxation. Vocalization and urination or defecation was also seen in 3 animals. Vehicle microinjections did not elicit changes in body temperature or behavior.

The hypothermic response to intracisternal administration of THC resulted in a more rapid onset and reached a greater maximum than that produced by ventricle III administration.

Intravenous administration of 500 mcg of THC produced significantly less hypothermia than intracisternal or intraventricular administration. Vehicle produced no significant alteration in body temperature.

Thermosensitive anterior hypothalamic neurons (pre-optic region) have been studied in cats anesthetized with either urethane or chloralose in an attempt to characterize the hypothermic action of THC at the neuronal level. One hundred and seventy-eight single neurons were isolated and subjected to thermal challenge, 66 were found to reproducibly alter firing frequency at a significant level (thermosensitivity, T.S., 0.75). Twenty-one of these units met the criteria for primary thermodetectors, 34 were heat-sensitive interneurons and 11 were cold-sensitive interneurons. Administration of THC (1.0-2.0 mg/kg i.v.) decreased the spontaneous firing and increased the T.S. of the primary thermodetector units. THC also increased the spontaneous firing frequency as well as the T.S. of heat-sensitive interneurons, while decreasing both the T.S. and spontaneous firing of cold-sensitive interneurons. The decreased spontaneous firing of primary thermodetectors could result from altered facilitory or inhibitory influences converging on these cells. The increased thermosensitivity is consistent with the hypothesis that the preoptic region modulates cannabinoid-induced hypothermia (7).

In preliminary studies, a high percentage (70%) of the units we have isolated in the medulla exhibited some thermosensitivity to local thermal challenge. Of the medullary units exhibiting some thermosensitivity, 47% demonstrated thermosensitive convergence to heating and/or cooling of spinal cord and/or preoptic region. The firing rate response to such convergent driving varies from neuron to neuron. Selected, stable thermosensitive units were challenged by systemic administration of small doses (0.5-2.0 mg/kg) of THC. Typically, THC decreases the spontaneous firing rate while the T.S. was differentially altered. Nonthermosensitive neurons (controls), exhibited little or no change in firing patterns after THC. These studies document the presence of thermosensitive neurons in the medulla. Many of these units receive convergent information from other known thermosensitive sites. These preliminary unit studies add support to our contention that the caudal brainstem is the principal site of action for THC induced hypothermia.

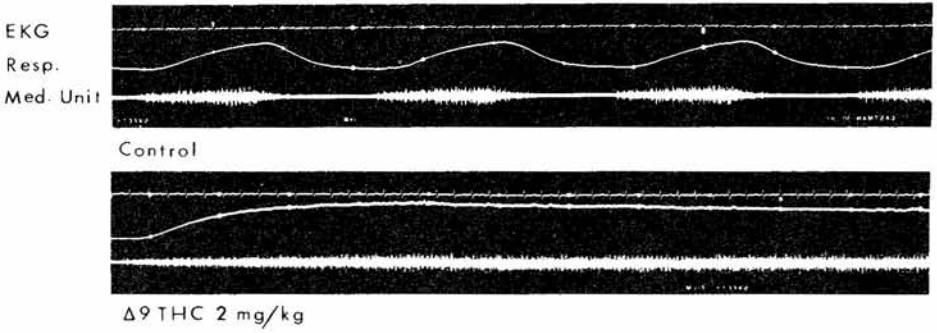
In anesthetized cats, THC has a marked depressant effect on spontaneous respiration. Figure 7 illustrates microelectrode recordings from medullary inspiratory neurons in two anesthetized animals. Note that the "inspiratory unit" begins firing immediately before the onset of inspiration and terminates firing prior to the onset of expiration. After i.v. THC administration, frank apneusis is evident in animal RC-15, while a shift toward apneustic respiration is demonstrated by animal RC-20. After THC the inspiratory neurons continue to fire for extended periods with concomitant maintenance of inspiration.

Doses of THC above 2 mg/kg reduce respiration rate by at least 50%. However, most striking, was the production of apneusis. Although this effect was most pronounced in animals anesthetized with chloralose urethane, it was also observed in the dial-urethane anesthetized animals. We frequently observed periods of apenusis with a duration in excess of 60 seconds. Onset of apneustic respiration ranged from 1 to 10 min after THC administration, and persisted for longer than 1 hour, often terminating in death. Coincident with reduced frequency of respiration and apneusis, arterial O_2 saturation was reduced by as much as 33%. Respiratory stimulants (doxapram, nikethamide, pentylenetetrazol) restored normal respiratory patterns for at least a brief period of time in every animal in which they were utilized. CNS depressants (diazepam, pentobarbital) increased both frequency and duration of apneustic periods. In cats pretreated with ethanol (500 mg/kg i.v.), a 1-mg/kg i.v. dose of THC induced marked apneusis in 5 of 6 cats studied.

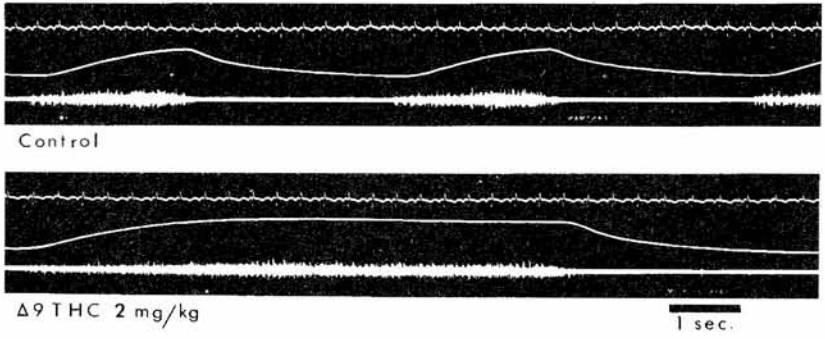
It was possible to induce apneusis with THC in cats with the neuraxis transected posterior to the colliculi. In two of

Δ9 THC on MEDULLARY "INSPIRATORY" NEURONS

THC-RC-15 ♂ 4·4 kg CU



THC-RC-20 ♂ 2·3 kg CU



these preparations, we were able to interrupt sustained inspiration by electrical stimulation of the "pneumotaxic center" (N. parabrachialis med.). Extracellular microelectrode recordings from medullary "inspiratory" and "expiratory" neurons were consistent with diaphragmatic EMG patterns.

Taken together, the data summarized here are consistent with and support our hypothesis that the primary autonomic effects of THC are mediated at caudal brainstem sites that are located for the most part between the levels of the midcolliculi and the first cervical segment.

ACKNOWLEDGMENT

The authors thank Mr. Michael Savio for his excellent technical assistance.

REFERENCES

1. Hosko, M. J., and Hardman, H. F., Evidence for a dual mechanism of action of cannabis on central cardiovascular control, in "Pharmacology of Marihuana" (M. C. Braude and S. Szara, eds.), pp. 239-253. Raven Press, New York, 1976.
2. Schmeling, W. T., and Hosko, M. J., Hypothermic effects of intraventricular and intravenous administration of cannabinoids in intact and brainstem transected cats, J. Neuropharmacol. 19:567-573 (1980).
3. Schmeling, W. T., Hosko, M. J., and Hardman, H. F., Potentials evoked in the intermediolateral column by hypothalamic stimulation-supression by delta-9-tetrahydrocannabinol, Life Sci. 29:673-680 (1981).
4. Schmeling, W. T., and Hosko, M. J., The effects of delta-9-tetrahydrocannabinol (THC) on centrally mediated homeostatic cardiovascular reflexes, Fed. Proc. 37:737 (1978).

FIGURE 7. Effect of THC on medullary respiratory neuron in two anesthetized, spontaneously breathing cats. The second trace in each panel (Resp.) goes up with inspiration. In both animals the post-THC records were obtained within 5 minutes of THc administration. The apneustic event illustrated in the second panel lasted longer than 90 seconds. The period of apneustic respiration in both animals had a duration longer than one hour.

5. Schmeling, W. T., and Hosko, M. J., Hypothermia induced delta-9-tetrahydrocannabinol in rats with electrolytic lesions of preoptic region, Pharmacol. Biochem. Behav. 5(1):79-83 (1976).
6. Hosko, M. J., Schmeling, W. T., and Hardman, H. F., Evidence for a caudal brainstem site of action for cannabinoid induced hypothermia, Brain. Res. Bull. 6:251-258 (1981).
7. Schmeling, W. T., and Hosko, M. J., Effect of delta-9-tetrahydrocannabinol on hypothalamic thermosensitive units, Brain. Res. 187:431-442 (1980).