European Journal of Pharmacology, 47 (1978) 303—310

CANNABINOID EFFECTS ON PLASMA CORTICOSTERONE AND UPTAKE OF ^{3}H-CORTICOSTERONE BY MOUSE BRAIN

KENNETH M. JOHNSON *, WILLIAM L. DEWEY **, KATHERINE S. RITTER and JACQUELINE S. BECKNER

Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, U.S.A.

Received 7 October 1977, accepted 14 October 1977

K.M. JOHNSON, W.L. DEWEY, K.S. RITTER and J.S. BECKNER, *Cannabinoid effects on plasma corticosterone and uptake of ^{3}H-corticosterone by mouse brain*, European J. Pharmacol. 47 (1978) 303—310.

The effects of three cannabinoids, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC), Δ^9-THC and cannabinol (CBN), ranging in behavioral activity from high to low, were studied on two aspects of pituitary—adrenal function. Plasma corticosterone levels were used as an index of adrenocorticotropic hormone (ACTH) release. All three cannabinoids elicited an increase in plasma corticosterone levels in a manner similar to their behavioral potency. These cannabinoids also elicited an increase in the concentration of ^{3}H-corticosterone taken up by the brains of adrenalectomized mice in a manner similar to their potency in elevating plasma corticosterone levels. The significance and possible underlying mechanism of the apparent correlation resulting between these effects and the behavioral effects of cannabinoids are discussed.

Structure—activity relationship Corticosteroids Hippocampus Cannabinoids
Adrenocorticotropic hormone

1. Introduction

(—)-trans-Δ^9-Tetrahydrocannabinol (Δ^9-THC), the major active cannabinoid in marihuana (Isbell et al., 1967, and Mechoulam, 1970), has been reported to activate the pituitary—adrenal axis as measured by adrenal ascorbic acid depletion (Dewey et al., 1970). This effect was verified by the demonstration that Δ^9-THC elevated plasma corticosterone levels (Kubena et al., 1971). Furthermore, this study indicated a hypothalamic or other central locus of this action of Δ^9-THC.

Several authors have noted that the effects of adrenocorticotropic hormone (ACTH) and Δ^9-THC on certain behavioral and pharmacological parameters are similar (for review, see Miller and Drew, 1974). For example, both ACTH and Δ^9-THC facilitate the acquisition of active avoidance tasks as well as reduce body temperature in laboratory animals. In addition, clinical observation of patients with adrenal hyperfunction and of those treated with high doses of ACTH indicate occurrence of emotional changes such as euphoria and depression (Schildkraut et al., 1968). Similar emotional changes have been reported to occur after use of marihuana (Schultes, 1969).

However, it is not difficult to find incongruities in the proposed correlation between the effects of Δ^9-THC and ACTH. For example, Dupont et al. (1970) have demonstrated a

* Present address of K.M.J.: Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.
** Address for reprints: William L. Dewey, MCV Box 726, Richmond, Virginia 23298, U.S.A.

positive correlation between elevated plasma corticosterone levels (dependent on ACTH release) and passive avoidance learning as well as an inverse correlation between plasma corticosterone levels and active avoidance learning. Δ^9-THC, however, elevates plasma corticosterone levels while having no effect on passive avoidance (Miller et al., 1973) while it facilitates active avoidance in rats (Barry and Kubena, 1971).

Inferential comparisons of this type are made difficult by the fact that ACTH and corticosterone often have opposite effects on the same behavioral system (Weiss et al., 1969; Endroczi, 1972). In addition corticosterone is believed to act directly on the brain to regulate the release of ACTH from the pituitary. Furthermore, lesion and electrical stimulation studies have suggested that the hippocampus is an integral part of a counterbalanced system regulating ACTH release (Mangili et al., 1966).

In view of the fact that the hippocampus selectively accumulates ^{3}H-corticosterone by a high-affinity, low capacity binding system (McEwen et al., 1969) and the recent claim that Δ^9-THC selectively altered the hippocampal and septal uptake of ^{3}H-corticosterone (Drew and Slagel, 1973) we have chosen to examine the effects of three cannbinoids on plasma corticosterone concentration as well as on the accumulation of ^{3}H-corticosterone by mouse brain. The three cannabinoids used in this study were selected on the basis of their relative behavioral activity. 11-OH-Δ^9-THC is a major metabolite of Δ^9-THC and is generally considered to have the same potency (Kosersky et al., 1974; Perez-Reyes et al., 1972) or greater potency than Δ^9-THC (Christensen et al., 1971; Lemberger et al., 1973). Cannabinol (CBN) is generally thought to have little or no behavioral activity (Christensen et al., 1971; Mechoulam and Edery, 1973). It should be noted, however, that CBN does possess considerable anticonvulsant activity, but even in this regard is about one-third as potent as Δ^9-THC (Karler et al., 1973).

2. Materials and methods

2.1. Animals

Male ICR mice of 20—30 g body weight were used. They were housed in groups of 6 in a room in which the temperature was maintained between 21 and 23°C. A daily light—dark cycle was maintained, with the lights on between 7.00 and 19.00 h. The mice had free access to food and water.

2.2. Treatments

Mice were removed from the animal room and placed in the experimental laboratory at least 2 h prior to the start of the experiment. Every attempt was made to handle the mice as gently and quietly as possible during the course of the experiment. In spite of these attempts to eliminate potential stressful conditions, we found day to day variations in plasma corticosterone levels of vehicle injected control mice. Therefore, the effects of each dose of each cannabinoid tested was compared to vehicle controls run at the same time.

In this series of experiments at least 6 mice were injected s.c. with either vehicle, which consisted of Emulphor (EL-260), ethanol, and saline (Cradock et al., 1973) or the desired cannabinoid given in a volume of 0.1 ml/10 g body weight. The mice were decapitated 30 min later and blood was collected from the trunk wound into heparinized glass tubes. After separation of plasma by centrifugation, the plasma was either processed immediately or stored at −20°C until analyzed (usually within 24 h).

In another series of experiments we compared the effects of cannabinoids on the brain regional distribution of tritium after an i.v. injection of corticosterone-1,2,^{3}H(N) (60 Ci/mmol, New England Nuclear Corporation, Boston, Mass.). In order to minimize competition between the exogeneously administered ^{3}H-corticosterone and the endogenous glucocorticoid (McEwen et al., 1969), bilaterally adrenalectomized (ADX) mice were used. Bilateral adrenalectomies were carried out on

mice anesthetized with methoxyflurane using a dorsal approach. ADX mice were maintained on 1.5% NaCl and free access to food during the interim between surgery and testing. McEwen et al. (1969) have shown that ^{3}H-corticosterone is rapidly removed from the blood and the brain of ADX rats (half-life of approximately 3 h) and that hippocampal binding of ^{3}H-corticosterone is greater than cortical binding within 2 h after adrenalectomy. Furthermore, hippocampal binding of ^{3}H-corticosterone increased out to about 20 h and then remained at or slightly below this level thereafter. Therefore, we chose to examine the effects of cannabinoids on brain uptake of ^{3}H-corticosterone 24 h after adrenalectomy.

In these experiments either vehicle or the cannabinoid was administered (s.c.) 15 min prior to the injection (i.v.) of either 1 or 2 μCi ^{3}H-corticosterone (10% ethanolic benzene diluted 1 : 100 with 0.9% NaCl). The animals were sacrificed 60 min later by decapitation. The brains were quickly removed, rinsed in ice-cold 0.9% NaCl, blotted dry, and dissected into six areas according to a minor modification of the technique described by Glowinski and Iverson (1966): the cortical area anterior to a coronal section passing through the anterior commisure as well as the striatum located posterior to the same section was descarded. The remaining "whole brain" was dissected into six areas including the cerebellum, medulla oblongata plus pons, cortex, hypothalamus, hippocampus, and midbrain (including the thalamus and subthalamus). These areas were weighed and placed in Packard combustion cups and allowed to dry overnight. The samples were subsequently oxidized in a Packard TriCarb sample oxidizer (97% recovery) and tritium was estimated by liquid scintillation spectrometry using the external standard ratio technique for quench correction.

2.3. Determination of plasma corticosterone

Plasma corticosterone was assayed in triplicate 10 μl samples according to an adaptation (Bowman and DeLuna, 1969) of the competitive protein binding assay of Murphy (1967). Triplicates of standard corticosterone (Sigma Chemical Co., St. Louis, Mo.) Consisting of 0, 1, 2, 3, 4 and 5 ng were run with each experiment and produced a linear displacement of the ^{3}H-corticosterone bound to the assay protein.

2.4. Source of cannabinoids

11-OH-Δ^9-THC, Δ^9-THC, and CBN were obtained from Dr. Robert Willette of the National Institute on Drug Abuse.

2.5. Statistics

The Student's *t*-test was used to compare the means of different treatment groups. In the two experiments which examined the dose–response of Δ^9-THC, overall comparisons between treatment groups were made by analysis of variance. Individual comparisons between means was accomplished by using Dunnett's modification of the *t*-test which uses the overall error estimate from the analysis of variance (Winer, 1972). In addition, analysis of variance and covariance was used to determine whether or not an interaction between dose and brain area existed regarding the distribution of ^{3}H-corticosterone (University of California, Los Angeles, Health Sciences Computing Facility).

3. Results

3.1. Effect of cannabinoid administration on plasma corticosterone concentration

Results presented in table 1 demonstrate that administration of 30 mg/kg Δ^9-THC resulted in maximal elevation of plasma corticosterone levels. This effect peaked 30 min after administration, and corticosterone levels were still elevated at 60 and 90 min, but not at 120 min (data not shown). A 30 min post-injection interval was chosen

TABLE 1

Levels of plasma corticosterone 30 min after the administration of various doses of 3 cannabinoids. Each cannabinoid was tested in 6 mice, with 6 mice in each vehicle control group. The vehicle control means ranged from 7.54 to 19.92 μg corticosterone/100 ml plasma. The mean ± S.E.M. of the 12 vehicle groups represented here is 11.92 ± 1.10 μg%.

Dose (mg/kg)	% Control ± S.E.M.		
	11-OH-Δ^9-THC	Δ^9-THC	CBN
3	382.1 ± 47.4 [3]	150.8 ± 37.3	130.1 ± 51.9
10	313.2 ± 15.1 [3]	182.9 ± 52.8 [1,4]	132.1 ± 39.5
30	345.4 ± 27.3 [3]	255.2 ± 28.4 [3]	221.8 ± 68.8 [2]
100	—	158.4 ± 20.5 [2]	200.9 ± 30.0 [2]
200	—	—	154.9 ± 24.6 [2]

[1] $p < 0.1$; [2] $p < 0.05$; [3] $p < 0.01$ (Student's t-test).
[4] In two additional experiments 10 mg/kg Δ^9-THC produced plasma corticosterone levels which were 187.2 and 161.0% of control, each being significant at the $p < 0.1$ level.

to compare the effects of several doses of 11-OH-Δ^9-THC, Δ^9-THC and CBN on plasma corticosterone levels. These data are presented in table 1. 11-OH-Δ^9-THC produced approximately a 2 to 3 fold increase in plasma corticosterone levels at each dose tested. Δ^9-THC produced a biphasic elevation of corticosterone levels, apparently peaking at 30 mg/kg. Cannabinol also elevated corticosterone levels in a biphasic dose—response fashion. The relative potency of these cannabinoids, at least at 3, 10 and 30 mg/kg, appears to coincide with their potency in altering behavior and other parameters, (including body temperature and catecholamine synthesis, Bloom et al., 1976) i.e. 11-OH-Δ^9-THC > Δ^9-THC > CBN.

3.2. Effect of Δ^9-THC on the net accumulation and regional distribution of ^{3}H-corticosterone in the brain of ADX mice

Δ^9-THC pretreatment resulted in a dramatic increase in the quantity of tritium found in the brains of mice which were injected with 1 μCi of ^{3}H-corticosterone 60 min prior to decapitation ($F_{4,31} = 10.4$, $p < 0.01$). Due to the expected, large inter-animal variability, only 30 and 100 mg/kg produced significant increases. These data are shown in fig. 1.

The regional distribution of the ^{3}H-corticosterone found in the brains of these mice was examined by comparing the concentration of tritium in each area to the concentration of tritium found in the whole brain. The effect of several doses of Δ^9-THC on this ratio for six brain areas is shown in fig. 2. Compari-

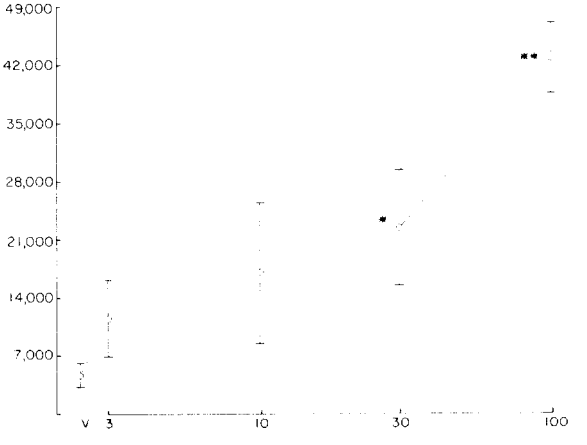

Fig. 1. Effect of Δ^9-THC on the uptake of ^{3}H-corticosterone by "whole brain" (see section 2.2.). Values are expressed as the mean ± S.E.M. (dpm/g whole brain). *$p < 0.025$, **$p < 0.005$ (Dunnett's t-test). Ordinate: dpm/g whole brain; abscissa: dose of Δ^9-THC (mg/kg).

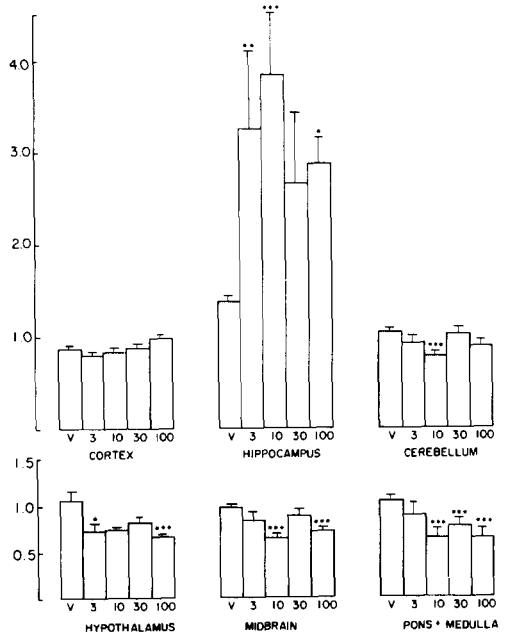

Fig. 2. Effect of Δ^9-THC on the uptake of ^{3}H-corticosterone by several brain areas. Uptake is expressed as dpm/g of a particular area relative to dpm/g in whole brain (mean ± S.E.M.). V = vehicle; numbers under each bar represent the dose (mg/kg) of Δ^9-THC. $*p < 0.05$; $**p < 0.025$; $***p < 0.01$ (Dunnett's t-test). Ordinate: (dpm/g area)/(dpm/g whole brain).

son of brain areas by analysis of variance revealed that the hippocampus retained more ^{3}H-corticosterone than all other areas after vehicle pretreatment ($F_{5,59} = 8.7$, $p < 0.01$), and is in agreement with the demonstration that the hippocampus possesses a high-affinity binding site for corticosterone (McEwen et al., 1969/1970). Fig. 2 also demonstrates that the Δ^9-THC-induced increase of ^{3}H-corticosterone uptake is unevenly distributed throughout the brain. Analysis of variance and covariance of this data revealed a significant difference between areas ($F_{5,155} = 48.7$, $p < 0.01$) and a significant interaction between the dose of Δ^9-THC and the different areas ($F_{20,155} = 3.6$, $p < 0.01$). Analysis of variance in individual brain areas indicated that Δ^9-THC administration significantly increased the concentration (relative to whole brain) of ^{3}H-corticosterone in the hippocampus ($F_{4,31} = 3.6, p < 0.025$), but decreased the relative concentration in the hypothalamus ($F_{4,31} = 2.9$, $p < 0.05$), midbrain ($F_{4,31} = 4.7$, $p < 0.01$), and pons-medulla ($F_{4,31} = 4.9$, $p < 0.01$). The relative concentration of ^{3}H-corticosterone in the cortex and cerebellum was not significantly altered by Δ^9-THC.

Since it is possible that an increase in concentration in one area relative to whole brain might tend to diminish the concentration relative to whole brain in other areas, we also calculated the concentration of ^{3}H-corticosterone in each area relative to that found in the cortex. Analysis of variance of this data yielded qualitatively similar results to those described above, except that the decreased concentration of ^{3}H-corticosterone in the hypothalamus failed to reach statistical significance.

3.3. Comparison of the effects of several cannabinoids on the net accumulation of ^{3}H-corticosterone in the brains of ADX mice

Two experiments were carried out to determine if any correlation existed between the potencies of the cannabinoids in elevating plasma corticosterone levels and their potential alteration of whole brain accumulation of ^{3}H-corticosterone. The first of these experiments compared the effect of 11-OH-Δ^9-THC to that of CBN. From the data presented in table 2, it can be discenred that 11-OH-Δ^9-THC significantly increased the concentration of ^{3}H-corticosterone found in the brain, while the concentration found after CBN was not significantly different from the vehicle controls. The second of these experiments compared the effects of all three cannabinoids used in this study. As can be seen, the increase in concentration of ^{3}H-corticosterone found in the brains of ADX mice treated with 10 mg/kg of 11-OH-Δ^9-THC, Δ^9-THC, and CBN roughly corresponds with the effect of these cannabinoids (at 10 mg/kg) on elevating plasma corticosterone concentrations (table 1).

TABLE 2

The effect of 3 cannabinoids on the uptake and retention of tritium by mouse brain after an i.v. administration of ^{3}H-corticosterone. 6 adrenalectomized mice in each group were pretreated with either vehicle or 10 mg/kg of the indicated cannabinoid 15 min prior to the administration of 2 μCi of ^{3}H-corticosterone. The mice were sacrificed 60 min later.

Experiment number	Vehicle	11-OH-Δ^9-THC	Δ^9-THC	CBN
1	11,824 ± 3,631	38,229 ± 10,576 [1]	—	6,706 ± 1,400
2	15,637 ± 2,059	33,116 ± 5,837 [2]	27,185 ± 7,190	22,808 ± 4,350

[1] $p < 0.05$; [2] $p < 0.025$ (Student's t-test).

4. Discussion

This study has demonstrated that 11-OH-Δ^9-THC, Δ^9-THC and CBN significantly increase plasma corticosterone levels when administered to mice. The relative potency of these cannabinoids in elevating plasma corticosterone levels coincides with the potency of these cannabinoids in altering several neurochemical, physiological and behavioral parameters (Harris et al., 1976), i.e. 11-OH-Δ^9-THC is more potent than Δ^9-THC and Δ^9-THC is more potent than CBN. Therefore, it is possible that some of the effects of cannabinoids may be mediated by the actions of corticosterone. Since plasma corticosterone levels are closely related to ACTH secretion, it is also possible that some of the effects of cannabinoids may be mediated by the actions of ACTH.

This study has also demonstrated that Δ^9-THC administration increased the amount of ^{3}H-corticosterone retained by the brains of ADX mice. Although the concentration of ^{3}H-corticosterone was increased in all brain areas examined after Δ^9-THC administration, the concentration found in the hippocampus was approximately three times greater than that in whole brain when analyzed after Δ^9-THC pretreatment as compared to about 1.4 times the whole brain concentration after vehicle pretreatment. We also observed that Δ^9-THC pretreatment resulted in very slight, but significant, decreases in the concentration of ^{3}H-corticosterone in midbrain, pons-medulla, and hypothalamus relative to the whole brain concentrations. In addition we observed that 11-OH-Δ^9-THC pretreatment also increased the concentration of ^{3}H-corticosterone found in whole brain, and, in fact, was more potent than Δ^9-THC and CBN in this regard. Thus it is possible that the effects of the cannabinoids on plasma corticosterone levels and uptake and/or retention of corticosterone by the brain are causally related.

The observations which we have reported here are somewhat contradictory to a report that 3 and 9 mg/kg Δ^9-THC stimulated and inhibited, respectively the uptake of ^{3}H-corticosterone by the hippocampus, septum and cortex of ADX rats (Drew and Slagel, 1973). A potential source for this discrepancy is that these investigators administered both Δ^9-THC and ^{3}H-corticosterone at approximately the same time and by the same route (i.p.). In view of the structural and physical similarities between the two compounds it is possible that the absorption and distribution of either agent could be altered by the other. Other possibilities included difference in species and time of sacrifice.

The mechanism by which 11-OH-Δ^9-THC and Δ^9-THC increased ^{3}H-corticosterone uptake by the brain is unknown at this time. Although the hippocampus is known to accumulate ^{3}H-Δ^9-THC, approximately one-half of the seventeen areas examined by

McIsaac et al. (1971) accumulated as much ^{3}H-Δ^9-THC as did the hippocampus. Δ^9-THC and corticosterone are also known to be predominately bound by different plasma proteins (Wahlqvist et al., 1970; Daughaday, 1958). In spite of these dissimilarities in distributions mechanisms, the structural and physical similarities of these two compounds suggest that a direct molecular interaction cannot be rules out as a potential contributor to this effect.

Given the apparent correlation between cannabinoid-induced elevations of plasma corticosterone levels and increased hippocampal binding of ^{3}H-corticosterone, it is pertinent to discuss the results of other experiments which may bear on this observed relationship. Pfaff et al. (1971) demonstrated that hippocampal neurons decreased and increased their firing rates in response to peripheral injections of corticosterone and ACTH, respectively. Thus, Δ^9-THC, by increasing the level of corticosterone in hippocampus, could indirectly produce a decrease in hippocampal firing rates. If the hippocampus does indeed exert an inhibitory influence on ACTH release, as proposed by others (Knigge and Hays, 1963; Yates et al., 1971), then the inhibition of hippocampal firing rates by the accumulated corticosterone could conceivably lead to an increase in ACTH release and consequent production of adrenal corticosterone. However, since Δ^9-THC administration produced altered concentrations of ^{3}H-corticosterone in areas other than the hippocampus as well, we cannot rule out the participation of these areas as sites which could influence ACTH release. As a matter of fact, Steiner (1970) has identified neurons in the hypothalamus and midbrain which are inhibited by dexamethasone and excited by ACTH. Contrary to the findings of Pfaff et al. (1971), Steiner found no steroid sensitive neurons in the dorsal hippocampus.

In conclusion, this study has demonstrated a correlation between cannabinoid-induced alterations in whole brain uptake of ^{3}H-corticosterone and increased ACTH release (as measured by plasma corticosterone levels). The fact that the rank-order potency of the three cannabinoids tested in altering these parameters of brain—pituitary—adrenal function is similar to previously published experiments regarding the relative potency of these cannabinoids in altering other neurochemical, physiological, and behavioral variables argues against the possibility that the observations reported here are due to the non-specific effects of stress produced by the administration of these cannabinoids. Although this study has not identified the specific interaction between the cannabinoids and ACTH-corticosterone regulation, it does suggest that corticosterone and/or ACTH may be specifically involved in the overall action of the cannabinoids.

Acknowledgements

This research was supported in part by U.S. Public Health Service Grants DA-00326, DA-00490 and F-32-DA-05012.

References

Barry, H., III and R.K. Kubena, 1971, Repeated high doses of Δ^1-tetrahydrocannabinol enhance acquisition of shock avoidance by rats, Proc. 79th Ann. Conv. Amer. Psychol. Assoc., 6, 747.

Bloom, A.S., K.M. Johnson, F.J. Bowman and W.L. Dewey, 1976, Correlation between the effects of several cannabinoids on catecholamine biosynthesis, body temperature and behavior, Federation Proc. 35, 506.

Bowman, R.E. and R.F. DeLuna, 1969, Protein-binding assays for adrenocorticoids, Behav. Res. Meth. Instrum. 1, 135.

Christensen, H.D., R.J. Freudenthal, J.T. Gidley, R. Rosenfield, G. Boegli, L. Testino, D.R. Brine, C.D. Pitt and M.E. Wall, 1971, Activity of Δ^8- and Δ^9-tetrahydrocannabinol and related compounds in the mouse, Science 172, 165.

Cradock, J.C., J.P. Davignon, C.L. Litterst and A.M. Guarino, 1973, An intravenous formulation of Δ^9-tetrahydrocannabinol using a non-ionic surfactant, J. Pharm. Pharmacol. 25, 345.

Daughaday, W.H., 1958, Binding of corticosteroids by plasma proteins, Arch. Intern. Med. 101, 286.

Dewey, W.L., T.C. Peng and L.S. Harris, 1970, The effect of 1-trans-Δ^9-tetrahydrocannabinol on the hypothalamo-hypophyseal-adrenal axis of the rat, European J. Pharmacol. 12, 383.

Drew, W.G. and D.E. Slagel, 1973, Δ^9-THC: Selective impairment of corticosterone uptake by limbic structures of the rat, Neuropharmacol. 12, 909.

Dupont, A., E. Endroczi and C. Fortier, 1970, in: The Influences of Hormones on the Central Nervous System, ed. D.H. Ford (Karger, Basel) p. 451.

Endroczi, E., 1972, in: Hormones and Behavior, ed. S. Levine (Academic Press, New York) p. 173.

Glowinski, J. and L.I. Iverson, 1966, Regional studies of catecholamines in the rat brain, J. Neurochem. 13, 655.

Harris, L.S., W.L. Dewey and R.K. Razdan, 1976, Cannabis, its chemistry, pharmacology, and toxicology, in: Handbook of Experimental Pharmacology, Section 7, Chapter 1 (Springer-Verlag, New York).

Isbell, H., C.W. Gorodestzsky and F. Korte, 1967, Effects of (−)-Δ^9-trans-tetrahydrocannabinol in man, Psychopharmacologia 11, 184.

Karler, R., W. Celey and S.A. Turkanis, 1973, The anticonvulsant activity of cannabidiol and cannibinol, Life Sci. 13, 1527.

Knigge, K.M. and M. Hays, 1963, Evidence of inhibitive role of hippocampus in neural regulation of ACTH release, Proc. Soc. Expts. Biol. Med. 114, 67.

Kosersky, D.S., D.E. McMillan and L.S. Harris, 1974, Δ^9-Tetrahydrocannabinol and 11-hydroxy-tetrahydrocannabinol: Behavioral effects and tolerance development, J. Pharmacol. Exptl. Therap. 189, 625.

Kubena, R.K., J.L. Perhach and H. Barry III, 1971, Corticosterone elevation mediated centrally by Δ^1-tetrahydrocannabinol, European J. Pharmacol. 14, 89.

Lemberger, L., R. Martz, B. Rodda, R. Forney and H. Rowe, 1973, Comparative pharmacology of Δ^9-tetrahydrocannabinol and its metabolite, 11-OH-Δ^9-tetrahydrocannabinol, J. Clin. Invest. 52, 2411.

Mangili, G., M. Motta and L. Martini, 1966, Control of adrenocorticotropic hormone secretion, in: Neuroendocrinology, Vol. 1, eds. L. Martini and W.F. Ganog (Academic Press, New York) p. 297.

McEwen, B.S., J.M. Weiss and L.S. Schwartz, 1969, Uptake of corticosterone by rat brain and its concentration by certain limbic structures, Brain Res. 16, 227.

McEwen, B.S., J.M. Weiss and L.S. Schwartz, 1970, Retention of corticosterone by cell nuclei from brain regions of adrenalectomized rats, Brain Res. 17, 471.

McIsaac, W.M., G.E. Fritchie, J.E. Idanpaan-Heikkila and B.T. Ho, 1971, Distribution of marihuana in monkey brain and concomitant behavioural effects, Nature 230, 593.

Mechoulam, R., 1970, Marihuana chemistry, Science 168, 1159.

Mechoulam, R. and H. Edery, 1973, Structure—activity relationships in the cannabinoid series, in: Marihuana (Academic Press, New York) p. 103.

Miller, L.L. and W.G. Drew, 1974, Cannabis: Neural mechanisms and behavior, in: Marihuana, Effects on Human Behavior, ed. L.L. Miller (Academic Press, New York) p. 177.

Miller, L.L., W.G. Drew and P. Joyce, 1973, Δ^9-THC: Effect on acquisition and retention of a one-trial passive avoidance response, Behav. Biol. 8, 421.

Murphy, B.E.P., 1967, Some studies of the protein-binding steroids and their application to routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay, J. Clin. Endocrinol. 27, 973.

Perez-Reyes, M., M.C. Timmons, M.A. Lipton, R.H. Davis and M.E. Wall, 1972, Intravenous injection in man of Δ^9-tetrahydrocannabinol and 11-OH-tetrahydrocannabinol, Science 177, 633.

Pfaff, D.W., M.T.A. Silva and J.M. Weiss, 1971, Telemetered recording of hormone effects on hippocampal neurons, Science 172, 394.

Schildkraut, J.H., J.M. Davis and G.L. Clearman, 1968, in: Psychopharmacology: A Review of Progress, ed. D.H. Efron (U.S. Public Health Service Publication, no. 1836) p. 625.

Schultes, R.E., 1969, Hallucinogens of plant origin, Science 163, 245.

Steiner, F., 1970, Effects of ACTH and corticosteroids on single neurons in the hypothalamus, Prog. Brain Res. 32, 102.

Wahlqvist, M., I.M. Nilsson, F. Sandberg, S. Agurell and B. Grandstand, 1970, Binding of Δ^1-tetrahydrocannabinol to human plasma proteins, Biochem. Pharmacol. 19, 2579.

Weiss, J.M., B.S. McEwen, M.T. Silva and M. Kalkut, 1969, Pituitary—adrenal influences on fear responding, Science 163, 197.

Winer, B., 1972, Statistical Principles in Experimental Design (McGraw-Hill Book Company, New York).

Yates, F.E., S.M. Russell and J.W. Maran, 1971, Brain-adenohypophyseal communication in mammals, Ann. Rev. Physiol. 33, 393.