Neuropharmacology, 1973, 12, 909–914 Pergamon Press. Printed in Gt. Britain.

PRELIMINARY NOTE

Δ^9-THC: SELECTIVE IMPAIRMENT OF CORTICOSTERONE UPTAKE BY LIMBIC STRUCTURES OF THE RAT*

W. G. DREW and D. E. SLAGEL

Laboratories of Behavioral Neurophysiology, Department of Psychiatry, Department of Surgery, Division of Neurosurgery, Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40506

(*Accepted* 20 *March* 1973)

Summary—Δ^9-Tetrahydrocannabinol (THC) has a marked influence on the selective uptake of ^{3}H-corticosterone by the hippocampus and septum of the rat. Analysis of variance on blood/tissue ratios showed that a dose of 9 mg/kg of Δ^9-THC significantly ($P < 0{\cdot}01$) lowered ^{3}H-corticosterone uptake in the hippocampus. Compared with uptake in frontal and hind cortices, Δ^9-THC selectively depressed ^{3}H-corticosterone uptake within the septum and hippocampus. The decrease in uptake across the 3 and 9 mg/kg doses for the limbic system components compared with the cortical samples is highly significant ($P < 0{\cdot}001$) and suggests that Δ^9-THC exerts differential actions on hormone uptake within the central nervous system.

Adrenal cortical hormones (e.g. corticosterone) have marked effects on the excitability of brain tissue, possibly through alterations in the metabolism of intra- and extracellular electrolytes (VALKANA, VERNADAKIS and TIMIRAS, 1967; VALKANA and TIMIRAS, 1968) and chemical transmitters (AZMITIA and MCEWEN, 1972; JAVOY, GLOWINSKI and KORDON, 1968; MAAS, 1972). There is evidence to suggest that these hormones may be accumulated in nervous tissue by an active uptake mechanism (HENKIN, CASPER, BROWN, HARLAN and BARTTER, 1968) which may involve the selective regional distribution of corticosterone binding proteins (MCEWEN, MAGNUS and WALLACH, 1972; STEVENS, GROSSER and REED, 1971; CHYTIL and TOFT, 1972). MCEWEN, WEISS and SCHWARTZ (1969) have reported that ^{3}H-corticosterone is selectively accumulated in the hippocampus and septum of the adrenalectomized rat, the accumulation being markedly elevated in these two structures relative to plasma and other brain areas. The uptake of labelled corticosterone is greater in neuronal elements than in glial and other tissue within the rat brain (FORD, RHINES and STIEG, 1971). Radioautography revealed that the hormone accumulated in neuronal cell bodies of the hippocampus (GERLACH and MCEWEN, 1972).

In view of the hypothesis that marihuana or Δ^9-tetrahydrocannabinol (Δ^9-THC) may selectively impair certain aspects of hippocampal neural functioning (DREW, KIPLINGER, MILLER and MARX, 1972) it was of interest to ascertain the potential of Δ^9-THC to inhibit, or alter, the selective uptake of ^{3}H-corticosterone by the hippocampus.

*This work was supported in part by U.S.P.H.S. Grant No. MH 18392.

METHODS

Ninety day-old, male hooded rats (Blue Spruce) which had been adrenalectomized (21 days post-operative) were employed in order to eliminate the competition for uptake with endogenous corticosterone (MCEWEN *et al.*, 1969). Each rat received a complete bilateral adrenalectomy (dorsal approach) under ether anaesthesia. Great care was taken to ensure complete extirpation of the adrenals without damage to their structure. The hooded rat is a strain having a low incidence of accessory adrenal tissues. The rats were housed singly and were maintained (under standard conditions of temperature and lighting) on a 0·9% saline drinking supply. Food was available *ad lib.*

Δ^9-Tetrahydrocannabinol suspended in 1% Tween 80-water (DREW, MILLER and WIKLER, 1972) was administered intraperitoneally (i.p.) at three dose levels—0, 3 and 9 mg/kg to three separate groups of adrenalectomized rats. These doses are within a range of doses known to have mild to pronounced effects on a variety of behaviors (DREW *et al.*, 1972; GRUNFELD and EDERY, 1969; MASUR, MARTY and CARLINI, 1971) without producing a general incapacitation of the rat. A fourth group received 0·9% saline (i.p.) in a volume equal to a mid-range THC injection volume. Immediately after receiving the THC or control injection, each rat was injected i.p. with 100 μCi of (1, 2, 6, 7-^{3}H)-corticosterone (New England Nuclear, 84·6 Ci/mM) dissolved in ethanol (100 μl). The experiment was conducted sequentially (drug injections, sacrifice and beginning of extraction) between 9 and 12 a.m. thereby eliminating the influence of diurnal rhythms.

The rats were decapitated 1 hr after injection, their brains rapidly removed and the areas of interest dissected. Tissues were weighed (0·001 g), minced and extracted by shaking in 3–9 ml of dichloromethane for 18 hr (MCEWEN *et al.*, 1969). Measurement of residual radioactivity in the minced tissue by combustion in a TriCarb Sample/Oxidizer showed 95% of the radioactivity had been extracted. One ml aliquots were mixed with 15 ml of Packard Permafluor (5 g/l of 2,5-diphenyloxazole (PPO) and 0·1 g/l of 1,4-bis-2-(4-methyl-5-phenyl-oxazoyl) benzene (dimethyl POPOP)) and counted at 23% efficiency (A.E.S. ranged from 0·5105 to 0·5337) in a Packard Model 3375 scintillation spectrophotometer.

In confirmation of MCEWEN *et al.* (1969) pilot studies revealed that ^{3}H-corticosterone was selectively accumulated in the hippocampus and septum to a much greater extent than other brain areas (brainstem, frontal, occipital and temporal cortices and thalamus-midbrain). Consequently, in the present investigation uptake was measured only in the hippocampus, septum, frontal and hind cortices.

RESULTS

Tetrahydrocannabinol had a marked influence on the uptake of corticosterone by the septum and hippocampus. As may be observed in Figure 1, the 0 mg/kg dose level (Tween) had no effect on uptake of label when compared to the saline treated group. The 3 mg/kg dose level resulted in higher actual dis/min per mg wet wt values across all areas with the greatest value observed in the hippocampus and septum. Analysis of variance on blood/tissue ratios for each of the areas investigated showed that the highest dose level of THC blocked corticosterone uptake by the septum ($F = 3{\cdot}87$, $df = 13,10$; $P < 0{\cdot}05$), hippocampus ($F = 8{\cdot}32$, $df = 13,10$; $P < 0{\cdot}01$), frontal cortex ($F = 4{\cdot}75$, $df = 13,10$; $P < 0{\cdot}05$) and hind cortex ($F = 5{\cdot}76$, $df = 13,10$; $P < 0{\cdot}05$).

In order to assess the selectivity of this Δ^9-THC effect, dis/min per mg wet wt values for the septum and hippocampus were combined as were the values for the frontal and hind cortices in rats exhibiting comparable blood levels of corticosterone. An arbitrary range of 100–270

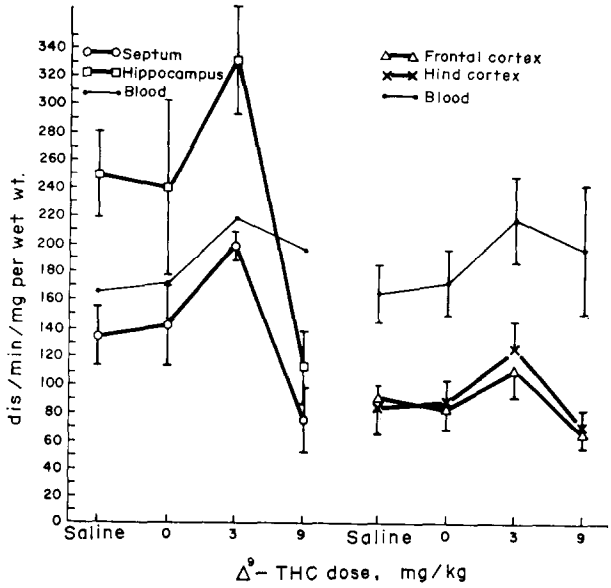

Fig. 1. ^{3}H-corticosterone uptake (dis/min per mg wet wt) in selected limbic and neocortical components of rat brain under 0, 3 and 9 mg/kg dose levels of Δ⁹-THC. Each point represents a mean value obtained from 5 rats while the vertical bars (on half-bars) denote standard errors. For clarity hippocampal and septal values are plotted on the left while neocortical values are to the right.

dis/min per mg of blood was employed. Differences in activity at the 3 mg/kg dose level compared to the 9 mg/kg dose level were then evaluated by the *t*-test. Even though activities were somewhat elevated under the 3 mg/kg treatment this elevation did not attain significance. However, the depression in label uptake at the higher dose level was significant ($P < 0{\cdot}001$—2 tail test).

DISCUSSION

The results of the present investigation suggest that corticosterone uptake by the hippocampus and septum may be differentially affected by Δ⁹-THC. Low doses of Δ⁹-THC tend to increase while high dose levels reliably impair the uptake of corticosterone by the hippocampus and septum. To our knowledge, this represents the first demonstration of a direct, non-inferential action of a cannabinoid on neural and/or neuroendocrine functions of either the hippocampus or septum.

Currently the hippocampus and septum seem to represent major target structures for corticosterone within the central nervous system. Both structures have been suspected for some time to play a role in the feedback regulation of the hypothalamo-pituitary-adrenal axis (for review see KENDALL, 1971). The finding that Δ⁹-THC may impair the uptake of corticosterone by the hippocampus seems to represent a consequence of acute intoxication heretofore unrecognized. This finding would appear to shed new light on the recent report by KUBENA, PERHATCH and BARRY (1971) that Δ⁹-THC causes an activation of the pituitary-adrenal axis in the rat, presumably by stimulation at a central site. Should the results of the present study be corroborated, the findings of KUBENA *et al.* (1971) might then be interpreted on the basis of a disfunction within an inhibitory rate-sensitive or level-sensitive neural

feedback loop (YATES, RUSSELL and MARAN, 1971). Accordingly, decreased corticosterone uptake (or decreased detection) by the hippocampus (or septum) in the presence of THC might falsely trigger the increased release of adrenocorticotrophic hormone (ACTH) by the pituitary, in turn increasing the production of corticosteroids. Since hippocampal lesions in rats result in chronic increases in ACTH levels (FENDLER, KARMOS and TELEGDY, 1961; KNIGGE and HAYS, 1963) and in view of the suspected inhibitive role of the hippocampus in the regulation of ACTH release (KNIGGE and HAYS, 1963) it becomes tempting to hypothesize that THC may induce a " pharmacological hippocampectomy ". The present data indicate a THC-induced disruption of ^{3}H-corticosterone uptake while the data of KUBENA *et al.* (1971) indicate increased corticosterone secretion probably reflective of increased ACTH release.

Adrenocorticotrophic hormone and related peptides exert certain behavioral effects which are similar to those produced by Δ^9-THC. It is well known that drugs which inhibit the extinction of behavioral responses will also induce dishabituated responding in habituated subjects (see: CARLTON, 1968; THOMPSON and SPENCER, 1966). We have recently demonstrated that low doses of Δ^9-THC will dishabituate the running response of rats habituated to activity wheels (DREW and MILLER, 1973). Adrenocorticotrophic hormone likewise inhibits the extinction of avoidance responses (MURPHY and MILLER, 1955; MILLER and OGAWA, 1962; DEWIED, 1966) as well as appetitively motivated behaviors (GRAY, 1971) independent of an action on the adrenal cortex (DEWIED, 1969). Moreover, ACTH facilitates the acquisition of avoidance tasks (see: DEWIED, 1969, 1970) as will Δ^9-THC (BARRY and KUBENA, 1971), however this issue is still unsettled (HENRIKSSON and JARBE, 1971).

Electrographic evidence suggests that Δ^9-THC may interfere with limbic neuroendocrine pathways. Hippocampal theta waves disappear under the influence of Δ^9-THC in both rats and rabbits at a time when EEG voltage output is reduced (LIPPARINI, DE CAROLIS and LONGO, 1969). Later the well known THC-induced spike-and-wave complexes appear which under high dose levels are almost continuous. These observations have been corroborated (MASUR and KHAZAN, 1970; PIRCH, COHN, BARNES and BARRATT, 1972). The effects of ACTH are strangely similar. KAWAKAMI, SETO, TERASAWA and YOSHIDA (1967) report changes in the EEG of the hippocampus following 0·5 units of ACTH. Both the amplitude and frequency of the theta waves were decreased while sporadic spindle-like bursts were observed in the amygdala.

Since the selective binding of corticosterone to the hippocampus and septum is well documented it is suggested that the THC-induced reduction of corticosterone uptake by the hippocampus and septum may be related in part to the behavioral effects of the drug. Taken together, the neuroendocrine, behavioral and electrographic data suggest the possiblity that certain effects of Δ^9-THC may be secondary to or dependent upon the increased release of ACTH.

Acknowledgements—We wish to thank Dr. RALPH E. MILLER for his advice and assistance on the adrenalectomy aspects of this study. We also gratefully acknowledge the skillful technical assistance of BARBARA UTZ, ELIZABETH BAUGH and PATRICIA JOYCE.

REFERENCES

AZMITIA, E. C., JR. and MCEWEN, B. S. (1972). Effect of adrenal secretion on midbrain tryptophan hydroxylase activity in rats. *Soc. for Neurosci.* 2nd Ann. Mtg. p. 226.

BARRY, H. III and KUBENA, R. K. (1971). Repeated high doses of Δ^1-tetrahydrocannabinol enhance acquisition of shock avoidance by rats. *Proc. 79th Ann. Conven. Am. Psychol. Ass.* pp. 747–748.

CARLTON, P. L. (1968). Brain acetylcholine and habituation. In: *Progress in Brain Research-Anticholinergic Drugs* (BRADLEY, P. B. and FINK, M., Eds.), pp. 48–60. Elsevier, New York.

CHYTIL, F. and TOFT, D. (1972). Corticoid binding component in rat brain. *J. Neurochem.* **19**: 2877–2880.

DEWIED, D. (1966). Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. *Proc. Soc. exp. Biol. Med.* **122**: 28–32.

DEWIED, D. (1969). Effects of peptide hormones on behavior. In: *Frontiers in Neuroendocrinology* (GANONG, W. F. and MARTINI, L., Eds.), pp. 97–140. Oxford University Press, New York.

DEWIED, D. (1970). Pituitary control of avoidance behavior. In: *The Hypothalamus* (MARTINI, L., MOTTA, M. and FRASCHINI, F., Eds.), pp. 1–8. Academic Press, New York.

DREW, W. G., KIPLINGER, G. F., MILLER, L. L. and MARX, M. (1972). Effects of propranolol on marihuana-induced cognitive dysfunctioning. *Clin. Pharmac. Ther.* **13**: 526–533.

DREW, W. G. and MILLER, L. L. (1973). Differential effects of Δ^9-THC on locomotor behavior in activity-wheel-habituated and nonhabituated rats. *Pharmacology* (In Press).

DREW, W. G., MILLER, L. L. and WIKLER, A. (1972). Effect of Δ^9-THC on the open-field activity of the rat. *Psychopharmacologia (Berl.)* **23**: 289–299.

FENDLER, K., KARMOS, G. and TELEGDY, G. (1961). The effect of hippocampal lesion on pituitary–adrenal function. *Acta physiol. hung.* **20**: 293–297.

FORD, D. H., RHINES, R. K. and STIEG, C. (1971). Hormone localization in the nervous system. In: *Influence of Hormones on the Nervous System* (FORD, D. H., Ed.), pp. 2–16. S. Karger, Basel.

GERLACH, J. L. and MCEWEN, B. S. (1972). Rat brain binds adrenal steroid hormones: radioautography of hippocampus with corticosterone. *Science* **175**: 1133–1136.

GRAY, J. A. (1971). Effect of ACTH on extinction of rewarded behavior is blocked by previous administration of ACTH. *Nature, Lond.* **229**: 52–54.

GRUNFELD, Y. and EDERY, H. (1969). Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. *Psychopharmacologia (Berl.)* **14**: 200–210.

HENKIN, R., CASPER, A. G., BROWN, R., HARLAN, A. C. and BARTTER, F. C. (1968). Presence of corticosterone and cortisol in the central and peripheral nervous system of the cat. *Endocrinology* **82**: 1058–1061.

HENRIKSSON B. G. and JARBE, T. (1971). Effects of diazepam on conditioned avoidance learning in rats and its transfer to normal state conditions. *Psychopharmacologia (Berl.)* **20**: 186–190.

JAVOY, F., GLOWINSKI, J. and KORDON, C. (1968). Effects of adrenalectomy on turnover of norepinephrine in the rat brain. *Eur. J. Pharmac.* **4**: 103–104.

KAWAKAMI, M., SETO, K., TERASAWA, E. and YOSHIDA, K. (1967). Mechanisms in the limbic system controlling reproductive function of the ovary with special reference to the positive feedback of progestin to the hippocampus. In: *Progress in Brain Research. Structure and Function of the Limbic System* (ADEY, W. R. and TOKIZANE, T., Eds.), pp. 69–102. Elsevier, New York.

KENDALL, J. W. (1971). Feedback control of ACTH secretion. In: *Frontiers in Neuroendocrinology* (GANONG, W. F. and MARTINI, L., Eds.), pp. 177–207. Oxford University Press, New York.

KNIGGE, K. M. and HAYS, M. (1963). Evidence of inhibitive role of hippocampus in neural regulation of ACTH release. *Proc. Soc. exp. Biol. Med.* **114**: 67–69.

KUBENA, R. K., PERHATCH, J. L., JR. and BARRY, H. III. (1971). Corticosterone elevation mediated centrally by Δ^1-tetrahydrocannabinol in rats. *Eur. J. Pharmac.* **14**: 89–92.

LIPPARINI, F., DE CAROLIS, A. S. and LONGO, V. G. (1969). A neuropharmacological investigation of some trans-tetrahydrocannabinol derivatives. *Physiol. Behav.* **4**: 527–532.

MAAS, J. W. (1972). Adrenocortical steroid hormones, electrolytes, and the disposition of the calecholamines with particular reference to depressive states. *J. psychiat. Res.* **9**: 227–241.

MASUR, J. and KHAZAN, N. (1970). Induction by cannabis sativa (marihuana) of rhythmic spike discharges overriding REM sleep electro corticogram in the rat. *Life Sci.* **9**: 1275–1280.

MASUR, J., MARTY, R. M. W. and CARLINI, E. A. (1971). Effects of acute and chronic administration of cannabis sativa and (−)Δ^9-trans-tetrahydrocannabinol on the behavior of rats in an open field. *Psychopharmacologia (Berl.)* **19**: 388–397.

MCEWEN, B. S., MAGNUS, C. and WALLACH, G. (1972). Soluble corticosterone-binding macromolecules extracted from rat brain. *Endocrinology* **90**: 217–226.

MCEWEN, B. S., WEISS, J. M. and SCHWARTZ, L. S. (1969). Uptake of corticosterone by rat brain and its concentration by certain limbic structures. *Brain Res.* **16**: 227–241.

MILLER, R. E. and OGAWA, N. (1962). The effect of adrenocorticotrophic hormone (ACTH) on avoidance conditioning in the adrenalectomized rat. *J. comp. physiol. Psychol.* **55**: 211–213.

MURPHY, J. V. and MILLER, R. E. (1955). The effect of adrenocorticotrophic hormone (ACTH) on avoidance conditioning in the rat. *J. comp. physiol. Psychol.* **48**: 47–49.

PIRCH, J. H., COHN, R. A., BARNES, P. R. and BARRATT, E. S. (1972). Effects of acute and chronic administration of marijuana extract on the rat electrocorticogram. *Neuropharmacology* **11**: 231–240.

STEVENS, W., GROSSER, B. I. and REED, D. J. (1971). Corticosterone-binding molecules in rat brain cytosols: regional distribution. *Brain Res.* **35**: 602–607.

THOMPSON, R. F. and SPENCER, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* **73**: 16–43.

VALKANA, T. and TIMIRAS, P. S. (1968). Effect of hypothyroidism on Na^+ K activated mg ATPase and ionic metabolism in the developing brain of the rat. *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**: 470.

VALKANA, T., VERNADAKIS, A. and TIMIRIS, P. S. (1967). Influence of estradiol and cortisol on electrolytes in the central nervous system of developing rats. *Neuroendocrinology* **2**: 326–329.

YATES, F. E., RUSSELL, S. M. and MARAN, J. W. (1971). Brain-adenohypophysial communication in mammals. *A. Rev. Physiol.* **33**: 393–444.