Pharmacology Biochemistry & Behavior, Vol. 15, pp. 779–783, 1981. Printed in the U.S.A.

Tetrahydrocannabinol Potentiates Reserpine-Induced Hypokinesia

D. E. MOSS,[1] S. B. McMASTER[2] AND JOSEPH ROGERS[3]

Department of Psychology, University of Texas at El Paso, El Paso, TX 79968

Received 18 July 1981

MOSS, D. E., S. B. McMASTER AND J. ROGERS. *Tetrahydrocannabinol potentiates reserpine-induced hypokinesia.* PHARMAC. BIOCHEM. BEHAV. **15**(5) 779–783, 1981.—Delta-9-tetrahydrocannabinol (THC), a substance in marihuana, was found to produce a profound potentiation of reserpine-induced hypokinesia in rats as measured with a bar test. In these experiments, THC had no hypokinetic effect by itself but produced a more than 20-fold increase in the hypokinesia produced by reserpine. Reserpine-induced hypokinesia has been viewed as animal model of Parkinson's Disease. THC potentiation of reserpine-induced hypokinesia was observed to be both time- and dose-dependent (1 to 10 mg/kg THC). When administered by gavage to reserpine-pretreated subjects (7.5 mg/kg IP, 24 hours before), THC produced a potentiation of hypokinesia that developed fully within 1 hour, lasted at least 5 hours, and was absent by 12 hours after THC administration. This THC effect was slightly increased by physostigmine, a cholinesterase inhibitor, relatively unaffected by scopolamine, a muscarinic antagonist, and almost completely blocked by ethopropazine, an anticholinergic antiparkinson drug. The effect was completely unaffected by naloxone. Insofar as reserpine has been used with some clinical efficacy in hyperkinetic movement disorders such as Huntington's disease and tardive dyskinesia, it may be that potentiation of reserpine's hypokinetic effect by a drug such as THC could greatly increase the clinical value of reserpine or related drugs in the treatment of these disorders.

Catalepsy Hypokinesia Akinesia Marihuana Tetrahydrocannabinol THC Huntington's disease Tardive dyskinesia Parkinson's disease

TETRAHYDROCANNABINOL (THC), along with other psychoactive constituents of marihuana, is reported to exert a variety of neurochemical effects, including actions on brain serotonin [15, 25, 26, 32], norepinephrine [15], dopamine [10,16], and acetylcholine [2, 7, 10, 22, 23, 33] metabolism. However, without clear animal models of marihuana and THC action, it remains uncertain which, if any, of THC's known pharmacological properties have functional significance, much less which, if any, underlie the compound's psychoactive effects. We describe here an animal model providing functional measures of THC action on the nervous system.

In order for such a model to be useful, a number of criteria need to be met. First, the paradigm should be based on a reliable, simple-to-observe behavior. Second, the effects of THC on that behavior should be dose- and time-dependent. Third, the behavioral measure should involve neural systems with at least partially-defined physiologic and pharmacologic correlates. This provides a starting point for meeting a fourth requirement, namely that THC should interact with known agonists and antagonists of the system in a pharmacologically consistent manner.

Reserpine has been reported to produce a reliable hypokinesia or cataleptic effect in rats that has been attributed to monoamine depletion [5] in the nigro-striatal system [1,24]. The behavioral rigidity and hypokinesia of reserpinized subjects has suggested a model for Parkinson's disease [6,28]. When injected intracranially [12], and in one case after oral administration [8], THC has also been reported to produce hypokinesia or catalepsy. It seemed possible, therefore, that reserpine-induced hypokinesia would be a behavior sensitive to THC administration.

The present experiments demonstrate a potent, easily observed effect of THC on reserpine-induced hypokinesia in rats. This effect is both dose- and time-dependent, and is more than 20-fold greater than that for THC or reserpine alone. Moreover, this action of THC is altered in a pharmacologically consistent manner by Parkinsonian agents presumed to act on nigro-striatal neurotransmission: the hypokinesia is potentiated by physostigmine, an anticholinesterase which exacerbates Parkinsonian symptoms; it is not significantly altered by naloxone or by scopolamine, an anticholinergic with little utility in treatment of Parkinson's disease; and it is significantly reversed by ethopropazine (Parsidol), an anticholinergic antiparkinsonian agent [3,31]. In addition to their relevance for studies on the pharmacology of marihuana and THC, these results strongly suggest that patients taking monoamine de-

[1]Send reprint requests to the first author at the above address.
[2]Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Science Center, Oklahoma City, OK 73104.
[3]A. V. Davis Center for Behavioral Neurobiology, The Salk Institute, P. O. Box 85800, San Diego, CA 92138.

pleting compounds or who exhibit symptoms of Parkinson's disease be specifically cautioned against the illicit use of marihuana or THC.

METHOD

Subjects

Male Sprague-Dawley rats (250–300 gm) raised from Holtzman stock in the University of Texas at El Paso animal colony served as subjects. These animals were maintained on ad lib chow and water. The lighting in the animal colony was regulated automatically to come on at 1900 hours and go off at 0700 hours (i.e., reverse cycle). The ambient temperature was maintained at 20–21 degrees Centigrade.

Hypokinesia Behavioral Test

Behavioral tests were conducted in a lighted room and began at 1300 hours. Subjects were placed in individual clear plastic observation chambers for a pretest period of 30 min. At the end of the pretest observation period, hypokinesia was measured using a bar test wherein the animal was placed in a standing position with its forepaws on a horizontal metal bar 9 cm above a metal platform. Both the metal bar and the metal platform were connected to an electronic touch sensor which operated a clock for automatically timing (to 0.1 sec) how long a subject stood at the bar. Rats were tested four at a time on four separate 12 cm wide bars. A maximum time of 1800 sec (30 min) was allowed. Each rat was tested under only one drug condition.

Drug Administration

Reserpine was obtained in the commercial preparation of 2.5 mg/ml (CIBA-GEIGY, NJ). A dose of 7.5 mg/kg (volume of 3 ml/kg) was injected IP only once between 1300 hours and 1500 hours the day before the behavioral test for hypokinesia. Fischer and Heller [9] have reported that the motor effects of reserpine develop shortly after injection, remain stable for 24 hours or more, than gradually diminish to normal after 48 hours. Rats were tested 22 to 24 hours after reserpine administration so that the effects would be stable at the time of testing.

Delta-9-tetrahydrocannabinol (THC) was supplied by the Research Technology Branch of NIDA as a 200 mg/ml solution in ethanol. The THC was prepared for gavage administration by diluting it 1:20 into olive oil, making a 10 mg/ml THC solution in olive oil with 5% ethanol. Five hours before the behavioral test, THC was administered in a dose of 10 mg/kg. Gavage was selected so that the THC could be reliably delivered, absorbed by a natural route, metabolized into whatever forms occur *in vivo*, and distributed into brain tissue through normal biological mechanisms. THC was given, in most experiments, five hours before the behavioral tests because, when administered by gavage, THC produces large and highly reliable decreases in rectal temperature which peak approximately four hours after administration, remain constant for about two hours thereafter, and then return to normal over the next six hours (Moss, unpublished experiments). In order to equalize the effects of stress, handling, and ethanol, control animals not receiving THC were given an equal volume (1 ml/kg) of olive oil containing 5% ethanol by gavage according to the same schedule as THC rats.

Effects of THC on Reserpine-Induced Hypokinesia.

THC and reserpine were administered and hypokinesia tested as described above. A total of 32 subjects were randomly assigned to one of four treatment conditions: THC plus reserpine, THC plus the reserpine vehicle, reserpine plus the THC vehicle, or THC and reserpine vehicles alone (N=8 per group). Data were analyzed by means of a 2-way ANOVA, with presence or absence of THC as one factor, and presence or absence of reserpine as the other factor.

Tests of THC Dose-Dependence

A total of 51 rats were reserpinized, given THC by gavage, and tested for hypokinesia as described above. However four different doses of THC were employed: 0.0 mg/kg, 1.0 mg/kg, 5.0 mg/kg, and 10.0 mg/kg. Data were analyzed by means of a 1-way ANOVA, with THC dose as the single factor.

Time-Dependence of THC Effects

A total of 16 rats were reserpinized, given 10 mg/kg THC by gavage, and tested for hypokinesia as described above. However, THC was administered either 1 hour, 2 hours, 12 hours, or 24 hours before hypokinesia testing (N=4 per group). The time of testing was held constant at 1300 hours to minimize variability due to circadian effects.

Interaction with Parkinsonian Drugs and with Naloxone

A total of 45 rats were reserpinized, given 10 mg/kg THC by gavage, and hypokinesia tested as described above. Reserpine was administered 24 hours and THC 2 hours before the behavior test. These subjects were then assigned to one of four drug treatments. The first group (N=9) received 2.0 mg/kg scopolamine hydrobromide IP 15 min before the behavior test. The second group (N=8) received 30 mg/kg ethopropazine IP 15 min before testing. The third group (N=4) received 1 mg/kg naloxone IP 15 min before testing. This dose of naloxone has been shown to reverse endorphin-induced catalepsy [4,17]. The fourth group received 0.5 mg/kg physostigmine 15 min before behavior testing. This amount produces 80% inhibition of rat brain acetylcholinesterase *in vivo* 15 min after IP administration (Moss, unpublished experiments). Because we suspected possible problems with ceiling effects in studying physostigmine potentiation of THC-reserpine hypokinesia, the anticholinesterase was administered at four different THC doses. The latter were the same as, and comparisons were made to, the doses used in studies of THC dose-dependency (see above).

RESULTS

Effects of THC on Reserpine-Induced Catalepsy

As expected, reserpine alone produces hypokinesia, with average time on the bar being 38.6±30.8 sec as compared to a 2.9±0.8 sec average for vehicle-alone control rats (Fig. 1). THC alone does not produce hypokinesia (mean time on the bar ±SEM=1.8±0.3 sec). However, giving THC to reserpinized rats produces profound hypokinesia more than 20-fold greater (921.9±174.7 sec) than that for reserpine alone (Fig. 1). Thus, the 2-way ANOVA reveals highly significant main effects of THC, $F=24.68$, $p<0.001$ and reserpine, $F=28.98$, $p<0.001$, plus a highly significant interaction between the two drugs, $F=24.82$, $p<0.001$. Computation of ω^2 [14] shows that these three effects account for 75.8% of the variance.

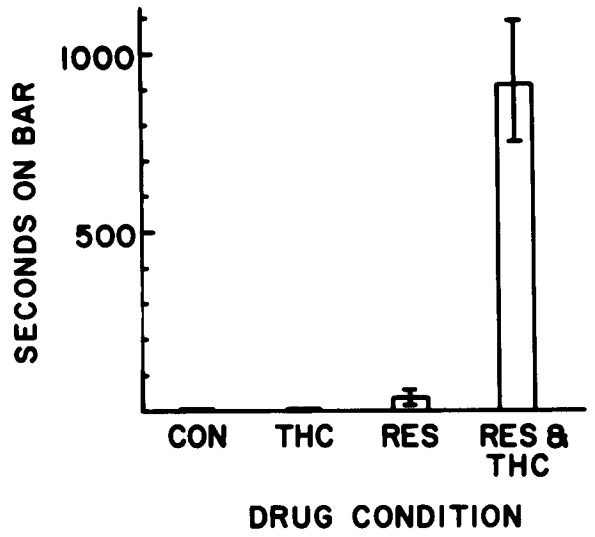

FIG. 1. Effect of THC on reserpine-induced catalepsy. The undrugged control animals (CON) had a mean time of 2.9 sec while the THC treated animals (THC) had a mean time of 1.8 sec. Comparison of the reserpine treated animals (RES) and THC/reserpine treated animals (RES and THC) shows the magnitude of the THC potentiation of reserpine-induced catalepsy. All groups contained 8 animals and the error bars represent one SEM.

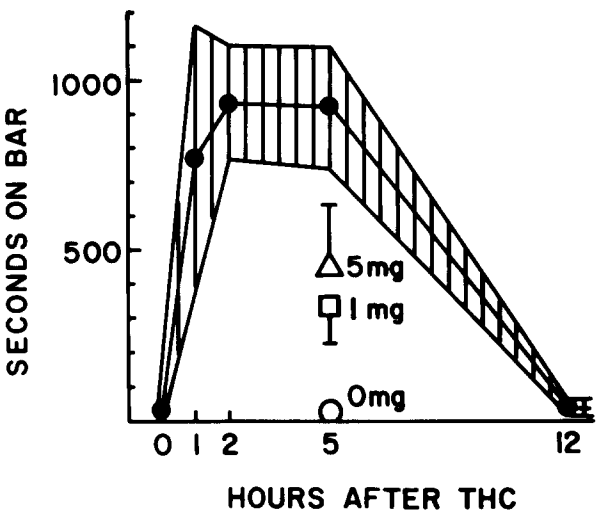

FIG. 2. Dose- and time-dependence of THC effects. The shaded area (mean±SEM) represents the effect of 10 mg/kg THC administered at various times before the tests for catalepsy. The open symbols at 5 hours show the effect of various doses of THC. Animals were tested only in one time or dose group. The results obtained at 24 hours were identical to those observed at 12 hours and are, therefore, not shown. All groups contained 4 animals except those at 5 hours which contained 8 animals.

Dose- and Time-Dependence of THC Effects

Figure 2 illustrates the hypokinetic effect of 10 mg/kg THC given by gavage to reserpinized rats 1, 2, 5, 12, or 24 hours before behavior testing. Potentiation of reserpine hypokinesia is significantly dependent on the time of THC administration, $F=5.78$, $p<0.01$. THC is fully effective within one hour of its administration, and remains so for at least five hours. By 12 hours there is no detectable effect of the drug (Fig. 2). Figure 2 also shows that THC potentiation of reserpine-induced hypokinesia is dose-dependent, $F=17.9$, $p<0.01$.

Interaction with Parkinsonian Drugs and with Naloxone

Neither naloxone nor scopolamine have any significant effect on THC-reserpine hypokinesia (Fig. 3). However, ethopropazine clearly reverses the THC effect, $F=17.6$, $p<0.001$, to a level comparable to that for the reserpine alone (Fig. 3). Physostigmine worsens THC-reserpine hypokinesia at all the THC doses tested. The effect, though small, is statistically reliable when compared to the data for THC-reserpine alone animals, $F=5.15$, $p<0.02$.

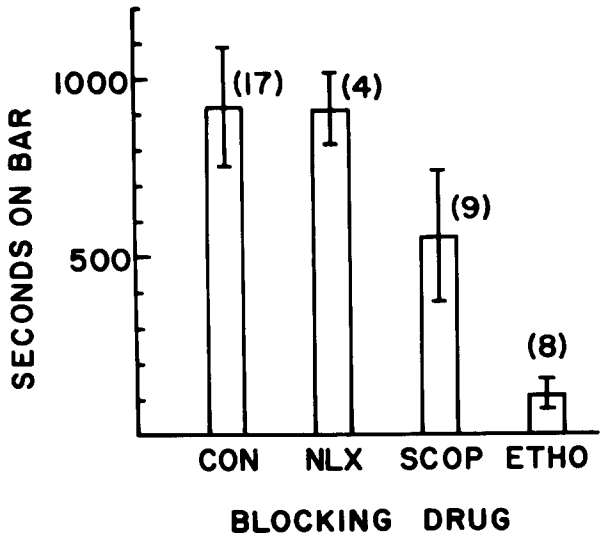

FIG. 3. Interactions with Parkinsonian drugs and naloxone. The blocking effects of naloxone (NLX, 1 mg/kg), scopolamine (SCOP, 2 mg/kg), and ethopropazine (ETHO, 30 mg/kg) on THC/reserpine catalepsy are shown in comparison with the control condition (CON) consisting of animals treated with THC and reserpine but which did not receive any other drug. The size of each group is shown in parentheses, and the error bars represent one SEM.

DISCUSSION

The present results do not pinpoint any precise mechanism by which the THC acts in the central nervous system. They do, however, provide a reliable, easily measured animal model and a starting point for understanding the pharmacology of THC. Reserpine-induced hypokinesia and rigidity mimic similar symptoms in Parkinson's disease [6,28]. The fact that THC profoundly increases these symp-

toms, plus the fact that such actions of THC are altered by various drugs in a manner consistent with their efficacy in treatment of Parkinson's disease, suggests that extrapyramidal nigro-striatal systems may be at least one site of THC action.

There are, of course, numerous neurotransmitters interactive with nigro-striatal function, including dopamine, serotonin, norepinephrine, acetylcholine, GABA, substance P, and met-enkephalin [13]. The failure of naloxone either to reverse or to potentiate THC-reserpine hypokinesia suggests a lack of involvement of opiate peptides. Because reserpine has its main pharmacological effect through depletion of monoamines [5], it is possible that THC is acting on a monoaminergic system to potentiate reserpine-induced hypokinesia. Alternatively, it is equally likely that the effect of THC is on another neurotransmitter system, and that this action is functionally revealed only after monoamine depletion.

Throughout this report, the term hypokinesia has been used instead of catalepsy; the latter is a term often used to describe reserpine's effects on locomotor behavior. The animals studied in the present experiments were not cataleptic in that they did not show the "waxy flexibility" usually associated with catalepsy. They had well organized righting reflexes and would struggle to right themselves when placed on their backs. The main effect of reserpine alone, or its combination with THC, was postural rigidity and an inability or unwillingness to initiate voluntary movements. This inability or unwillingness to initiate the voluntary movement required to get off the bar has been described as hypokinesia in our experiments. It should also be noted that the general appearance of animals treated with reserpine (i.e., hunched posture, ptosis, staring coat) was neither worsened by THC treatment nor lessened by further treatment with ethopropazine.

The results of the time-dependence experiment (Fig. 2) show that THC-potentiation of reserpine-induced hypokinesia develops rapidly, lasts up to 5 hours, and is over by 12 hours. This clear time course suggests that THC has some well defined neuropharmacological effect. It is also interesting to note that this time course is virtually identical to that reported for THC-induced psychoactive effects in humans after oral administration [18]. It seems possible, therefore, that an understanding of the neuropharmacology of THC potentiation of reserpine-induced hypokinesia could lead to an understanding of a basic mechanism of THC action.

In addition to their relevance for basic research into the mechanisms of THC action, the present findings may have preliminary clinical significance. THC's profound potentiation of extrapyramidal symptoms suggests that patients taking monoamine depleting drugs or suffering Parkinson's disease be specifically warned against illicit use of marihuana or THC. On a more positive note, however, it is possible that a THC analogue without euphoric side effects could be used to increase the therapeutic hypokinetic effect of reserpine in hyperkinetic motor disorders. Reserpine was one of the first agents to be employed successfully in the treatment of various hyperkinetic choreiform disorders [30], and it has been shown to be of benefit in tardive dyskinesia [27,29], and Huntington's disease [19, 20, 21]. Although clearly speculative at this time, it may be that a new THC analogue could increase the presently marginal effectiveness of reserpine and other drugs used in the treatment of a variety of hyperkinetic choreiform disorders.

ACKNOWLEDGEMENT

The technical assistance of Ms. Kim Vernon is gratefully acknowledged.

REFERENCES

1. Anden, N. E. and B. Johnels. Effect of local application of apomorphine to the corpus striatum and to the nucleus accumbens on the reserpine-induced rigidity in rats. *Brain Res.* **133:** 386–389, 1977.
2. Askew, W. E., A. P. Kimball and B. T. Ho. Effect of tetrahydrocannabinols on brain acetylcholine. *Brain Res.* **69:** 375–378, 1974.
3. Bianchine, J. R. Drugs for Parkinson's disease; centrally acting muscle relaxants. In: *The Pharmacological Basis of Therapeutics*, 6th ed., edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: MacMillan, 1980, pp. 475–493.
4. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* **194:** 630–634, 1976.
5. Brodie, B. B., J. S. Olin, R. Kuntzman and P. A. Shore. Possible interrelationship between release of brain norepinephrine and serotonin by reserpine. *Science* **125:** 1293–1294, 1957.
6. Carlsson, A. The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmac. Rev.* **11:** 490–493, 1959.
7. Domino, E. F. Effects of Δ^9-tetrahydrocannabinol and cannabinol on rat brain acetylcholine. In: *Marihuana: Chemistry, Biochemistry, and Cellular Effects*, edited by G. G. Nahas, W. D. M. Paton and J. E. Idanpaan-Heikkila. New York: Springer-Verlag, 1976, pp. 407–414.
8. Fairbairn, J. W. and J. T. Pickens. The oral activity of D^{-1} tetrahydrocannabinol and its dependence on prostaglandin E_2. *Br. J. Pharmac.* **67:** 379–385, 1979.
9. Fischer, E. and B. Heller. Pharmacology of the mechanism of certain effects of reserpine in the rat. *Nature* **216:** 1221–1222, 1967.
10. Friedman, E. and S. Gershon. Δ^9-tetrahydrocannabinol: Cerebral dopamine metabolism and behavioral effects after acute and chronic treatment in rats. In: *Proc. 5th Int. Cong. Pharmac.*, Abstr. 439, San Francisco, CA, 1972.
11. Friedman, E., I. Hanin and S. Gershon. Effect of tetrahydrocannabinols on ^{3}H-acetylcholine biosynthesis in various rat brain slices. *J. Pharmac. exp. Ther.* **196:** 339–345, 1976.
12. Gough, A. L. and J. E. Olley. Catalepsy induced intrastriatal injections of Δ^9-THC and 11-OH-Δ^9-THC in the rat. *Neuropharmacology* **17:** 137–144, 1978.
13. Hassler, R. Striatal control of locomotion, intentional actions and of integrating and perceptive activity. *J. neurol. Sci.* **36:** 187–224, 1978.
14. Hays, W. L. *Statistics for the Social Sciences*, 2nd ed. New York: Holt, Rinehart and Winston, 1973.
15. Holtzman, D., R. A. Lovell, J. H. Jaffee and D. X. Freedman. Δ^9-tetrahydrocannabinol: Neurochemical and behavioral effects in the mouse. *Science* **163:** 1464–1467, 1969.
16. Howes, J. and P. Osgood. The effects of Δ^9-tetrahydrocannabinol on the uptake and release of ^{14}C-dopamine from crude striatal synaptosomal preparations. *Neuropharmacology* **13:** 1109–1114, 1974.
17. Jacquet, V. F. and N. Marks. The C-fragment of β-lipotropin: An endogenous neuroleptic or antipsychotogen? *Science* **194:** 632–635, 1976.
18. Jaffe, J. H. Drug addiction and drug abuse. In: *The Pharmacological Basis of Therapeutics*, 6th ed., edited by A. G. Gilman and L. S. Goodman. New York: MacMillan, 1980, pp. 535–584.

19. Kempinsky, W. H., W. R. Boniface, P. P. Morgan and A. K. Busch. Reserpine in Huntington's chorea. *Neurobiology* **10:** 38–42, 1960.
20. Klawans, H. L. A pharmacologic analysis of Huntington's chorea. *Eur. Neurol.* **4:** 148–163, 1970.
21. Lazarte, J. A., M. C. Peterson, C. W. Baars and J. S. Pearson. Huntington's chorea: Results of treatment with reserpine. *Mayo Clin. Proc.* **30:** 358–365, 1955.
22. Luthra, Y. K. and H. Rosenkrantz. Cannabinoids: Neurochemical aspects after oral chronic administration on rats. *Toxic. appl. Pharmac.* **27:** 158–168, 1974.
23. Moss, D. E., P. L. Peck and R. Salome. Tetrahydrocannabinol and acetylcholinesterase. *Pharmac. Biochem. Behav.* **8:** 763–765, 1978.
24. Pijnenburgh, A. J. J. and J. M. van Rossum. Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. *J. Pharm. Pharmac.* **25:** 1003–1005, 1973.
25. Sofia, R. D., B. N. Dixit and H. Barry, III. The effect of Δ^9-tetrahydrocannabinol on serotonin metabolism in the rat brain. *Life Sci.* **10:** 425–436, 1971.
26. Sofia, R. D., R. J. Ertel, B. N. Dixit and H. Barry, III. The effect of Δ^9-tetrahydrocannabinol on the uptake of serotonin by rat brain homogenates. *Eur. J. Pharmac.* **16:** 257–259, 1971.
27. Soto, S., R. Daly and H. Peters. Reserpine therapy for phenothiazine-induced dyskinesia. *Dis. Nerv. Syst.* **32:** 680–685, 1971.
28. Steg, G. and B. Johnels. Motor functions of the striatum. In: *The Neostriatum*, edited by I. Divac, R. Gunilla and E. Oberg. New York: Pergamon, 1979, pp. 231–239.
29. Villeneuve, A. and A. Boszormenyi. Treatment of drug-induced dyskinesias. *Lancet* **1:** 353–354, 1970.
30. Weber, E. Rauwolfia-Alkaloid in der psychiatrie. Seine Wirkungsmoglichkeit mit Chlorpromazin. *Schweiz. med. Wschr.* **84:** 968, 1954.
31. Weiner, W. J. and H. L. Klawans. Cholinergic-monoaminergic interactions within the straitum: Implications for choreiform disorders. In: *Cholingeric-Monoaminergic Interactions in the Brain*, edited by L. L. Butcher. New York: Academic Press, 1978, pp. 335–362.
32. Welch, B. L., A. S. Welch, F. S. Messiha and H. J. Berger. Rapid depletion of adrenal epinephrine and elevation of telencephalic serotonin by (−)-trans-Δ^9-tetrahydrocannabinol in mice. *Res. Communs chem. Pathol. Pharmac.* **2:** 382–391, 1971.
33. Yoshimura, H., M. Fujiwara and S. Ueki. Biochemical correlates in mouse-killing behavior of the rat: Brain acetylcholine and acetylcholinesterase after administration of Δ^9-tetrahyrocannabinol. *Brain Res.* **81:** 567–570, 1974.