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Investigations on Behavioral Effects of an Extract of *Cannabis sativa* L. in the Rat

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Abstract. The behavioral responses of the rat to an extract of *Cannabis sativa* were examined after IP injection of 5, 15 and 30 mg/kg (expressed as Δ^9 tetrahydrocannabinol). The lowest dose of the extract induced stereotyped behavior (rhythmic head movements, intermittent gnawing and sniffing) together with hypersensitivity to stimuli and hyperthermia. The administration of higher doses of the extract resulted, initially, in similar behavioral effects but of greater intensity, followed by a cataleptic state alternating with atonic muscular prostration; rectal temperature was decreased. Pre-treatment with 6-hydroxydopamine (6-OHDA, which produces degeneration of catecholamine-containing nerve terminals) or pimozide (blocker of dopamine receptors) significantly reduced both stereotypy and hyperreactivity. Thermic effects were also antagonized by 6-OHDA pre-treatment. Cannabis-induced catalepsy was enhanced by pimozide but reduced by atropine (3 mg/kg SC). These results support the hypothesis that catecholamines play an important role in the complex behavioral effects of cannabis.

Key words: Cannabis – catecholamines – Hyperreactivity – Hyperthermia – Pimozide – Atropine – 6-OHDA – Stereotypy – Rat

Numerous attempts have been made to elucidate the mechanisms of action of cannabis and its active constituents. There are conflicting results concerning their interactions with neurotransmitters. For example, catecholamine levels have been reported to be raised (Constantidinis and Miras 1971) lowered (Schildkraut and Efron 1971; Graham et al. 1974) or unchanged (Maître et al. 1970; Taylor and Fennessy 1977) by Δ^9-tetrahydrocannabinol (Δ^9-THC) or cannabis extracts. It is also unclear whether a causal relationship exists between neurochemical changes induced by cannabis and behavioral parameters (Bloom and Kiernan 1980). These reports together with those from previous work in which we showed the involvement of a catecholaminergic mechanism in behavioral responses of the rabbit to another hallucinogenic drug, mescaline (Ferri et al. 1977), prompted us to study the effects of pharmacological manipulations on behavior of rats treated with an extract of *Cannabis sativa* L., rich in psychotropic constituents.

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Materials and Methods

Animals

Male Sprague-Dawley rats (200 – 220 g) were used and kept under standardized environmental conditions (room temperatures 20° ± 2°C, humidity 60%). Some rats were prepared for intracerebral administration with a cannula implanted in the lateral brain ventricle, as described by Altaffer et al. (1970). For each treatment, groups of ten rats were used. Drugs were administered either IP in a volume of 1 ml/kg or intracerebroventricularly in a volume of 20 µl/rat.

Drugs and Procedure

Extract of *Cannabis sativa* L. (supplied by Dr. G. Mazzone) was obtained by petroleum ether treatment, and contained 11.27% of Δ^9-THC as determined by gas-chromatography against a known concentration of 4-androstene-3,17-dione as internal standard (Fetterman et al. 1971). After distillation of the solvent, the residue was dissolved in olive oil and injected IP at doses of 5, 15 and 30 mg/kg (expressed as Δ^9-THC).

6-Hydroxydopamine HBr (6-OHDA, Hoffmann-La Roche, Basel) was dissolved in 20 µl of saline with ascorbic acid (1 mg/ml) and administered intracerebroventricularly twice (24-h interval) at a dose of 150 µg 3 days before the extract, in order to produce degeneration of catecholamine terminals and catecholamine depletion (Breese and Traylor 1970).

Pimozide (Janssen Pharmaceuticals, Beerse) a dopamine receptor blocker, was prepared as described by Cox and Lee (1977) and administered at a dose of 0.5 mg/kg/IP alone or 2 h prior to different doses of cannabis extract.

Atropine H_2SO_4 (C. Erba), an anti-cholinergic drug, was dissolved in saline and injected IP at a dose of 3 mg/kg alone or 10 min prior to different doses of cannabis extract.

Observations of Behavior

Stereotyped Behavior. The following scale was used to measure the intensity of the components of stereotyped behavior (sniffing, gnawing, rhythmic head movements):
absent = 0; occasional = 1; frequent = 2; constant = 3.

Hyperreactivity. The following scale was used to measure hyperreactivity (vocalization and/or starting) of a rat to a tactile stimulus (gentle pressure bilaterally exerted, behind the animal forelimb):
absent = 0; occasional = 1; frequent = 2; constant = 3.

Catalepsy. The time (min) for which a rat remained with front limbs over a 10-cm bar was taken as an estimate of the intensity of catalepsy, quantified by the following scale: 0 min = 0; up to 5 min = 1; up to 10 min = 2; > 10 min = 3.

Rectal temperature. This was monitored continuously by means of a thermistor probe (Ellab Instruments).

The behavior of animals receiving the antagonists pimozide or atropine alone was examined at 15-min intervals for the first 60 min and then every hour for a total of 4 h. Temperature was measured every hour for a total of 4 h.

In animals receiving cannabis alone or cannabis after the antagonist, behavioral responses were evaluated, at 10-min intervals, for the first 120 min following cannabis administration. Temperature was measured every hour for 4 h after cannabis.

Behavior was examined in 6-OHDA-treated rats for 3 days at 12-h intervals and then, after cannabis administration, for 2 h at 10-min intervals. Temperature was measured for 4 h after cannabis. The Mann-Whitney *U*-test was used for calculations of significance levels in various treatments (Siegel 1956). The occurrence (%) of each behavioral response was multiplied by its score (maximum $100 \times 3 = 300$) and represented by histograms.

Results

The smallest dose of the extract of *Cannabis sativa* L. (5 mg/kg Δ^9-THC) induced stereotyped behavior in the rat, rhythmic head movements, intermittent gnawing and sniffing being prevalent; hypersensitivity to touch also occurred. Increasing the dose resulted in the same effects but of greater intensity. The effects on behavior obtained with 30 mg/kg of extract within 2 h following administration are shown in Fig. 1. These effects occurred soon after cannabis administration, and then gradually disappeared, while a cataleptic state appeared to alternate with atonic muscular prostration of the animal. Catalepsy, fully evident in a 60 – 120 min interval following cannabis administration, reached a maximum with the highest dose of extract (Fig. 1). Rectal temperature (Fig. 2) exhibited a small but significant increase with the low dose of cannabis extract (5 mg/kg Δ^9-THC) whereas a marked and long lasting hypothermia was shown with the highest dose (30 mg/kg Δ^9-THC).

6-OHDA treatment had no effects on behavior in the time interval preceding cannabis administration. 6-OHDA pre-treatment significantly reduced stereotyped caused by cannabis extract and completely prevented hyperreactivity. Cannabis-induced catalepsy was less evident (Fig. 1) while a state of prostration prevailed. Thermic effects were also antagonized by 6-OHDA pre-treatment (Fig. 2).

Pimozide by itself did not affect behavior in the 3-h time interval following its administration; with the dose adopted, only a small cataleptogenic effect appeared at the end of the 4th hour of observation. Pimozide had no significant effect on temperature. Stereotyped behavior and hyperreactivity caused by cannabis extract were significantly reduced by pre-treatment with pimozide, whereas an increase in the intensity of catalepsy occurred (Figs. 1 and 2).

Atropine which by itself did not affect behavior produced a significant reduction in cannabis-induced stereotypy; catalepsy was almost completely absent (Fig. 1). Thermic effects, as well as hyperreactivity caused by the extract of cannabis, were not substantially modified by atropine.

Discussion

Administration of an extract of *Cannabis sativa* to the rat resulted in behavioral effects which were dose-related, reproducing essentially the results obtained in various laboratories with cannabis or its active constituents, cannabinoids (Grunfeld and Edery 1969; Lipparini et al. 1969; Domino 1971; Järbe and Henricksson 1973; Gough and Olley 1977; Bloom and Kiernan 1980). Behavioral effects of cannabis extract were strikingly modified by drugs that interact with the neurotransmitters dopamine and acetylcholine. In particular, the reduction of hyperreactivity and stereotypy (especially evident in the initial period following cannabis administration) in rats pre-treated with pimozide leads us to believe that this complex of symptoms is caused, at least in part, by facilitated dopaminergic transmission. We have confirmed, in our experiments, the low cateleptogenic effect of pimozide and the delayed onset of this effect (Niemegeers and Janssen 1979; McMillen et al. 1980). Since catalepsy appears late in the course of cannabis administration and is enhanced by pimozide, we suggest that impairment by cannabis of dopaminergic transmission follows an initial

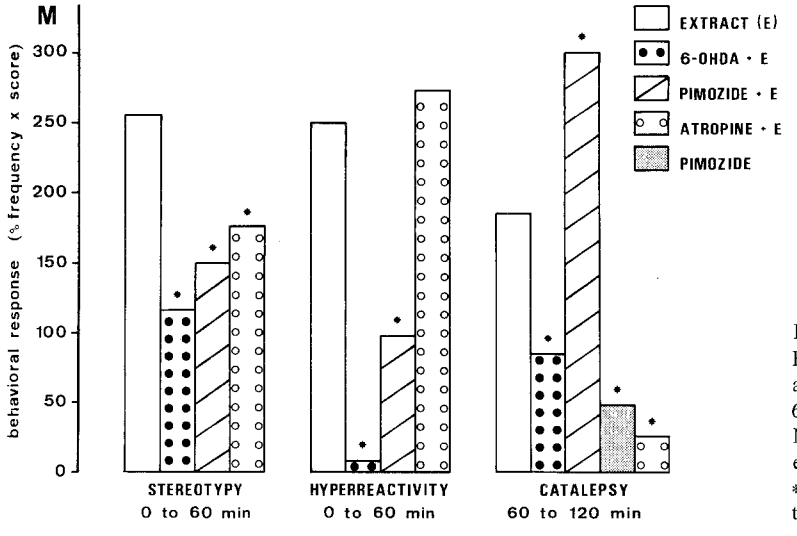

Fig. 1
Behavioral effects elicited by a cannabis extract (30 mg/kg IP as Δ^9-THC) in normal rats and in rats pre-treated with 6-hydroxydopamine (6-OHDA), atropine or pimozide. Measure of behavior (M) = sum of the frequency (%) of each response multiplied by its intensity (score).
* Analysis by Mann-Whitney *U*-test, $P < 0.01$ vs rats treated with cannabis only

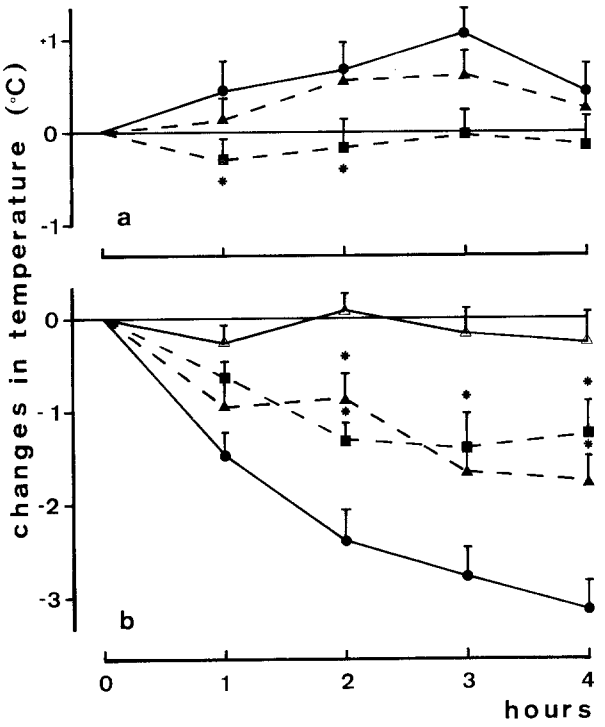

Fig. 2. Changes in rectal temperature in rats treated with: (**a**) (——) saline; (●——●) Cannabis extract (5 mg/kg IP Δ^9-THC); (■——■) 6-OHDA + extract (5 mg/kg IP Δ^9-THC); (▲——▲) Pimozide (0.5 mg/kg IP) + extract (5 mg/kg IP Δ^9-THC); (**b**) (——) saline; (●——●) Cannabis extract (30 mg/kg IP Δ^9-THC); (■——■) 6-OHDA + extract (30 mg/kg IP Δ^9-THC); (△——△) Pimozide (0.5 mg/kg IP); (▲——▲) Pimozide (0.5 mg/kg IP) + extract (30 mg/kg IP Δ^9-THC); Each point represents the mean of measurements in ten animals ± SEM $P < 0.05$ vs. animals treated only with cannabis extract

phase of facilitation. At the same time stimulation of cholinergic mechanisms may contribute to catalepsy, as well as stereotypy, as shown by its prevention with atropine. These findings, together with those reported by Gough and Olley (1975) indicating that catalepsy induced by Δ^9-THC, one of the most active psychoactive constituents of cannabis, is potentiated by extrapyramidal lesions, lead to the hypothesis that cannabis extract produces stereotypy and catalepsy by an effect on dopaminergic and cholinergic mechanisms in the basal ganglia. The reports of Shannon and Fried (1972) showing that Δ^9-THC is deposited largely in extrapyramidal structures, and of Domino (1971) showing an increase of brain acetylcholine by cannabinoid derivatives, further support this possibility. 6-OHDA was particularly effective in preventing the hypersensitivity of rats to stimuli. Since this drug reduced dopaminergic function as well as that of noradrenaline (Korstrzewa and Jacobowitz 1974), noradrenaline involvement is also plausible in the complex behavioral picture evoked by cannabis, an hypothesis in line with the recent study of Poddar and Dewey (1980). Moreover, from the findings of Järbe and Henricksson (1973) it may be inferred that a component of "distress" may participate in cannabis-induced hyperreactivity (vocalization), since this sign is prevented not only by the neuroleptic chlorpromazine, but also by the minor tranquilizer diazepam.

The biphasic effect we have shown on temperature with cannabis extract corroborates findings obtained with Δ^9-THC by Taylor and Fennessy (1977), who also proposed that this drug, depending on dose, may increase or decrease the release of brain serotonin, an important regulator of thermoregulatory functions, in the rat. It appears, however, from our experiments with antagonists, that catecholamines also play an important role in cannabis-induced changes of temperature, as suggested by Bloom and Kiernan (1980) who showed hypothermic effects of Δ^9-THC, in mice at ambient temperature, correlated with increases in catecholamines synthesis rate.

In contrast to our findings, Davies and Graham (1980) showed that the marked hypothermia produced in the mouse by oral administration of 20 mg/kg Δ^9-THC is potentiated by dopamine antagonists, haloperidol in particular. However, considerable evidence suggests that dosage, route of administration and species appear to be critical for pharmacological effects on temperature regulation (Cox and Lomax 1977). It is worthwhile noting that the dose of the dopamine antagonist pimozide used by us in the rat (unlike haloperidol in the mouse), did not significantly affect basal temperature, as already shown by Janssen et al. (1968).

Therefore, the present findings suggest a role for catecholamines in the mediation of the behavioral syndrome elicited by cannabis or its active constituents.

References

Altaffer FB, De Balbian Werster F, Hall S, Long CJ, D'Encarnacao P (1970) A simple and inexpensive cannula technique for chemical stimulation of the brain. Physiol Behav 5:119–121

Bloom AS, Kiernan CJ (1980) Interaction of ambient temperature with the effects of Δ^9-tetrahydrocannabinol on brain catecholamine synthesis and plasma corticosterone levels. Psychopharmacology 67:215–219

Breese GR, Traylor TD (1970) Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. J Pharmacol Exp Ther 174:413–419

Constantidinis J, Miras CJ (1971) Effect of hashish smoke sublimate on hypothalamic noradrenaline studied by the fluorescence method. Psychopharmacologia 22:80–90

Cox B, Lee TF (1977) Do central dopamine receptors have a physiological role in thermoregulation? Br J Pharmacol 61:83–86

Cox B, Lomax P (1977) Pharmacologic control of temperature regulation. Ann Rev Pharmacol Toxicol 17:341–357

Davies JA, Graham JDP (1980) The mechanism of action of Δ^9-tetrahydrocannabinol on body temperature in mice. Psychopharmacology 69:299–305

Domino EI (1971) Neuropsychopharmacologic studies of marihuana, some synthetic and natural THC derivatives in animals and man. Ann NY Acad Sci 191:166–191

Ferri S, Arrigo-Reina R, Spadaro C, Braga P (1977) 6-hydroxydopamine inhibits some effects of mescaline centrally administered to rabbits. Psychopharmacology 55:147–149

Fetterman PS, Keith ES, Waller CW, Guerrero O, Doorenbos NJ, Quimby MW (1971) Mississippi-grown Cannabis sativa L.: Preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part. J Pharm Sci 60:1246–1249

Gough AL, Olley JE (1977) Δ^9-Tetrahydrocannabinol and the extrapyramidal system. Psychopharmacology 54:87–99

Graham JDP, Lewis MJ, Williams J (1974) The effect of Δ^9-tetrahydrocannabinol on the noradrenaline and dopamine content of the brain and heart of the rat. Br J Pharmacol 52:446P

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

Janssen PAJ, Niemegeers CJE, Schellechens KHL, Dresse A, Lenaerts FM, Pinchard A, Schaper WKA, Van Neuten JM, Verbruggen FJ

(1968) Pimozide, a chemically novel, highly potent and orally long-acting drug. I. The comparative pharmacology of pimozide, haloperidol and chlorpromazine. Arzneim-Forsch 18:261–279

Järbe TUC, Henriksson BG (1973) Vocalization: a characteristic cannabis-induced behavior in the rat? Physiol Psychol 1:351–353

Korstrzewa RM, Jacobowitz DM (1974) Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 26:199–288

Lipparini F, Scotti de Carolis A, Longo VG (1969) A neuropharmacological investigation of some trans-tetrahydrocannabinol derivatives. Physiol Behav 4:527–532

Maître L, Stachelin M, Bein HJ (1970) Effect of an extract of cannabis and some cannabinols on catecholamine metabolism in rat brain and heart. Agents and Actions 1:136–143

McMillen BA, German DCG, Sanghera MK, Warnack W, Shore AP (1980) Pimozide: delayed onset of action at rat striatal pre- and postsynaptic dopamine receptors. J Pharmacol Exp Ther 215:150–155

Niemegeers CJE, Janssen PAJ (1979) A systematic study of the pharmacological activities of dopamine antagonists. Life Sci 24:2201–2216

Poddar MK, Dewey WL (1980) Effects of cannabinoids on catecholamines uptake and release in hypothalamic and striatal synaptosomes. J Pharmacol Exp Ther 214:63–67

Schildkraut JJ, Efron DH (1971) The effects of Δ^9-tetrahydrocannabinol on the metabolism of norepinephrine in rat brain. Psychopharmacologia 20:191–196

Shannon ME, Fried PA (1972) The macro- and microdistribution and polymorphic electroencephalographic effects of Δ^9-tetrahydrocannabinol in the rat. Psychopharmacologia 27:141–156

Siegel S (1956) Non parametric statistics for the behavioural sciences. McGraw-Hill, London

Taylor DA, Fennessy MR (1977) Biphasic nature of the effects of Δ^9-tetrahydrocannabinol on body temperature and brain amines of the rat. Eur J Pharmacol 46:93–99

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