Psychopharmacologia (Berl.) 22, 80—90 (1971)

Effect of Hashish Smoke Sublimate on Hypothalamic Noradrenaline Studied by the Fluorescence Method

J. Constantinidis

University of Geneva, Department of Psychiatry, Bel-Air, 1225, Switzerland
(Director: Prof. J. de Ajuriaguerra)

C. J. Miras

University of Athens, Department of Biological Chemistry, Greece
(Director: Prof. C. J. Miras)

Received March 25, 1971

Abstract. A histochemical study with the fluorescence method showed that administration of cannabis sublimate administered to rats increased the noradrenaline content of the terminal axonal varicosities of the hypothalamus, probably caused by a facilitation of noradrenaline uptake. The findings are discussed in relation to other known effects of cannabis derivatives, hypothermia, increased appetite and prolongation of amphetamine action.

Key-Words: Cannabis — Noradrenaline — Hypothalamus — Microscopy — Fluorescence.

Introduction

Some effects of cannabis in man and animals suggest an interaction with brain noradrenaline.

In animals, the anaesthesia after barbiturates is prolonged and the activity after amphetamine is increased by administration of hashish smoke sublimate (Miras, 1965), by cannabis extract (Garriott *et al.*, 1967), by natural and synthetic tetrahydrocannabinols (Garriott *et al.*, 1967; Kubena and Barry, 1970), and by synthetic potent analogues such as dimethyl-heptyl-pyran (Dagirmanjian and Boyd, 1962) or 3-hydro-6-dibenzopyran (Garriott *et al.*, 1968). Hashish smoking in man produces hypothermia and an increased appetite (Miras, 1965, 1969; Goodman and Gilmann, 1965). In animals intraperitoneal or intracerebral administration of hashish smoke sublimate also produces hypothermia (Miras, 1965; Garattini, 1965).

Other experiments have shown that amphetamine lowers the noradrenaline concentration and increases the serotonin concentration of the brain (Smith, 1965). Microinjection of noradrenaline in the hypothalamus produces hypothermia, while microinjection of serotonin produces hyperthermia (Feldberg and Myers, 1963, 1965); intrahypothalamic injection causes an increased food intake (Grossmann, 1962).

Holtzman *et al.* (1969) found that doses of 5 mg/kg up to 500 mg/kg tetrahydrocannabinol administered to mice increased the brain serotonin concentration from 10 to 20 %. The action on noradrenaline was found to be biphasic; 5—10 mg/kg significantly decreased the brain concentration, whereas 200—500 mg/kg increased it 20—45 %. One hour after 200 mg/kg tetrahydrocannabinol the increase of serotonin and noradrenaline was maximal and lasted about 24 h. The onset of hypothermia is rapid and follows the change in the brain amine concentration.

In rabbits, the effect of cannabis extract on the noradrenaline concentration differed in different parts of the brain (Miras, Kephalas, and Papadakis). After 300 mg/kg i.p. of cannabis extract the noradrenaline content of the cerebellum decreased 55 %, of the striatum plus the anterior part of hypothalamus decreased 22 %, while the posterio-superior part of hypothalamus plus the quadrigeminal area showed an increase of 80 %, and pons plus medulla oblongata an increase of 26 %.

The above-mentioned observations motivated us to investigate the action of cannabis on hypothalamic noradrenaline. The histochemical fluorescence method of Falck, Hillarp *et al.* (1962) was applied, in which noradrenaline may be localized as a green fluorescence in the perikaryon and axonal and terminal varicosities.

In the animal experiments we chose to imitate the condition of the human hashish smoker during the state of intoxication, and for this reason the sublimate of hashish smoke was used. Only substances contained in the smoke are absorbed by the hashish smoker (Joachimoglou, 1965; Vieira *et al.*, 1967) and smoking induces a more intense intoxication than eating or chewing the drug (Watt, 1965). Some significant differences are found between the action of cannabis extract and of chemically pure tetrahydrocannabinol, e.g. the latter induces prolonged depression of the blood pressure (Garriott *et al.*, 1967) in contrast to the former. Efron (1968) suggests the possibility that some other constituents of cannabis than tetrahydrocannabinol may influence the effect directly or indirectly, e.g. by prolonging the effect, or by occupying some of the receptor sites.

Materials and Methods

Sublimate of hashish smoke was made after the standardized production of resin indicated by Joachimoglou (1965).

39 Male albino rats, Wistar N.W., 6 to 10 weeks of age weighing 200—250 g were used.

For intraventricular injections the animals were implanted with a permanent cannula into the right lateral ventricle of the brain, according to the method of Hayden *et al.* (1966).

1st Experiment

Animals pretreated i.p. with reserpine (3 rats), reserpine with Nialamid (3 rats) or saline (3 rats) received a dose of hashish smoke sublimate in propylene glycol with added Tween i.p. Drugs were administered at the following times:

0 h 00 Reserpine 10 mg/kg i.p.;
1 h 00 Nialamid 250 mg/kg i.p.;
3 h 00 Hashish smoke sublimate 300 mg/kg i.p.;
4 h 00 Decapitation.

2nd Experiment

Animals pretreated with Nialamid i.p. (3 + 3 rats, with a further 3 + 3 as controls) received i.p. L-DOPA alone (3 + 3 rats) or L-DOPA with hashish smoke sublimate (3 + 3 rats). Drugs were given at the following times:

0 h 00 Nialamid 250 mg/kg i.p.;
2 h 00 L-DOPA 75 mg/kg i.p. hashish smoke sublimate 300 mg/kg i.p.;
3 h 00 Decapitation.

3rd Experiment

Animals (12 rats) received a single injection of noradrenaline (0.05 mg in 10 μl) intraventricularly after pretreatment i.p. with: a) saline (3 rats) or hashish smoke sublimate (3 rats), b) reserpine (3 rats) or reserpine with hashish smoke sublimate (3 rats). Drugs were administered as follows:

0 h 00 Reserpine 10 mg/kg i.p.;
3 h 00 Hashish smoke sublimate 300 mg/kg i.p., noradrenaline 0.05 mg intraventricularly;
4 h 00 Decapitation.

4th Experiment

Animals (3 rats) received 0,1 mg of hashish smoke sublimate in 10 μl intraventricularly and were sacrified after 1 h; they were compared with animals (3 rats) that received 10 μl of saline intraventricularly.

After decapitation under light ether anesthesia the brains were removed quickly, sectioned frontally in 5 blocks, deep-frozen in propane cooled in liquid nitrogen, and freeze-dried in vacuo at −35° for 7 days. After drying, the blocks were treated with formaldehyde gas at +80° for 1 h in a closed glass vessel. After this treatment the blocks were vacuum embedded in paraffin (for technical details see (Falck and Owman, 1965). Sections (10 μ) were placed on slides and mounted in Entellan. The blocks containing the hypothalamus were studied on the fluorescence microscope in serial sections with mounting 1/10.

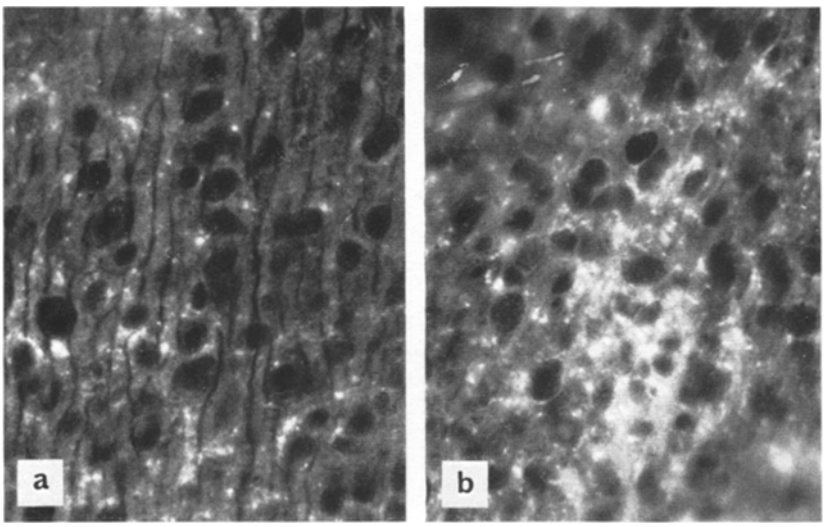

Fig. 1 a and b. Hypothalamic green varicosities of the rat in: a control; b 1 h after 300 mg/kg of hash. smok. subl. i.p. ×250

Results

The green varicosities in the hypothalamic nuclei around the third ventricle were studied by fluorescence microscopy. These varicosities containing noradrenaline are not found in the perikaryon of hypothalamic neurons, but in the terminal and preterminal parts of axons coming from neurons situated in the brain stem (Carlsson *et al.*, 1962; Anden *et al.*, 1965); they are related to the synaptic vesicles of the hypothalamus observed in electron-microscopy and which contain noradrenaline (de Robertis *et al.*, 1965).

a) After hashish smoke sublimate these hypothalamic green varicosities appear more numerous and with a stronger green fluorescence than in the controls (Fig. 1).

b) After reserpine treatment a depletion of green fluorescence in the varicosities of the hypothalamus is observed. This depletion is less noticeable if the reserpinised animal receives hashish smoke sublimate (Fig. 2). Nialamid administered after reserpine produces a very intense yellow fluorescence in serotonin neurons and varicosities of the brain stem, but the influence on the green fluorescence of catecholamine nerve cells in the brain stem and of noradrenaline varicosities of the hypothalamus is very slight (Dahlström and Fuxe, 1964); the green fluorescence of the hypothalamic varicosities is more intense in animals receiving

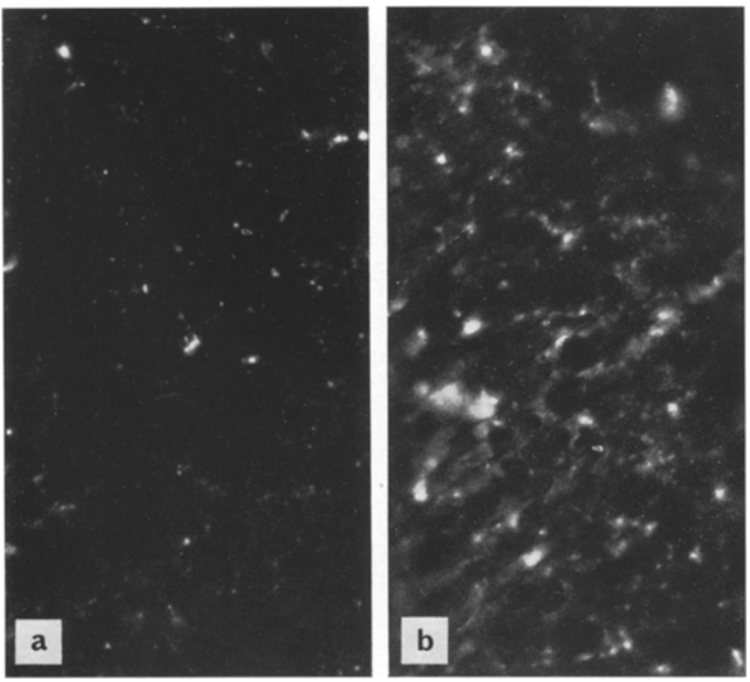

Fig. 2a and b. Hypothalamic green varicosities of the rat. a 4 h after 10 mg/kg of reserpine i.p.; b 4 h after 10 mg/kg of reserpine i.p. and 1 h after 300 mg/kg of hash. smok. subl. i.p. ×250

reserpine with Nialamid and hashish smoke sublimate than in those receiving reserpine with Nialamid alone.

c) Hashish smoke sublimate administered in the ventricle enhances an increase of green fluorescence in hypothalamic varicosities (Fig. 3).

d) Noradrenaline administered intraventricularly is taken up in the periventricular structures normally containing catecholamines. In reserpine treated animals noradrenaline (1—2 μg intraventricularly) is taken up if the monoaminoxidase has been inhibited; with higher exogenous amine concentrations accumulation of noradrenaline in the catecholamine neurons occurs after reserpine although the monoaminoxidase is not inhibited (Fuxe and Ungerstedt, 1968). With a high dose of noradrenaline (50 μg) administered intraventricularly, we observed an increase of green fluorescence in the hypothalamic varicosities and the dopamine neurons of the tubero-infundibular system with or without reserpine pretreatment; this phenomenon is accentuated by administration of hashish smoke sublimate (Fig. 4).

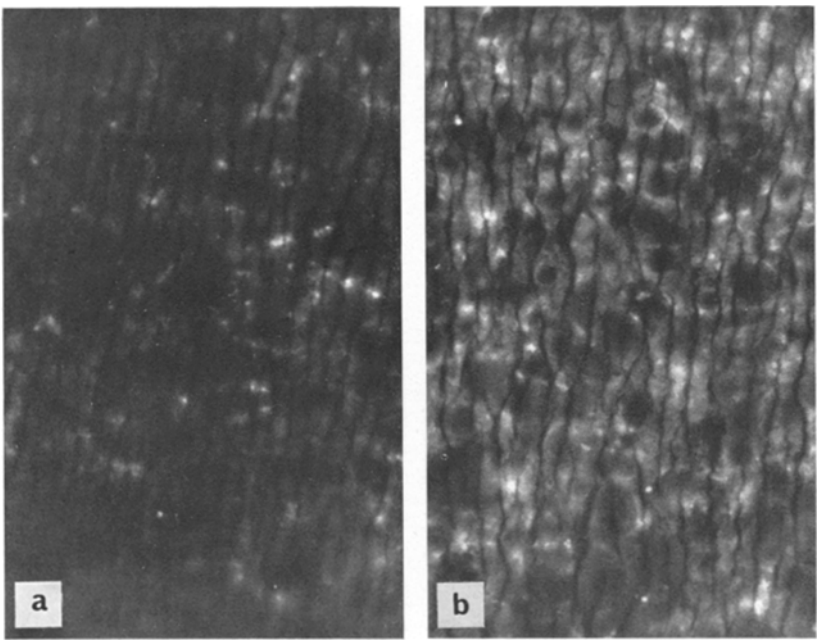

Fig. 3 a and b. Hypothalamic green varicosities of the rat 1 h after intraventricular administration of: a 10 µl saline; b 10 µl containing 0.1 mg of hash. smok. subl. ×250

e) L-DOPA administered i.p. produces a green fluorescence of the capillary endothelium of the brain, the L-DOPA being transformed in this endothelium to dopamine; this endothelial fluorescence is much stronger after Nialamid pretreatment and is abolished by the decarboxylase inhibitors (Bertler *et al.*, 1966; Constantinidis *et al.*, 1967). Administration of hashish smoke sublimate in animals treated with L-DOPA or Nialamid + L-DOPA has no influence on the intensity of the capillary green fluorescence.

Discussion

According to Holtzman *et al.* (1969) low doses of Cannabis derivatives decrease, and higher doses increase cerebral noradrenaline. Our experimental conditions correspond to his results with high doses as we observe an increase of green fluorescence in the noradrenaline containing varicosities of the hypothalamus, which means an increase of their noradrenaline content.

There are many possible mechanisms for such an increase: stimulation of synthesis, facilitation of uptake, decrease of catabolism.

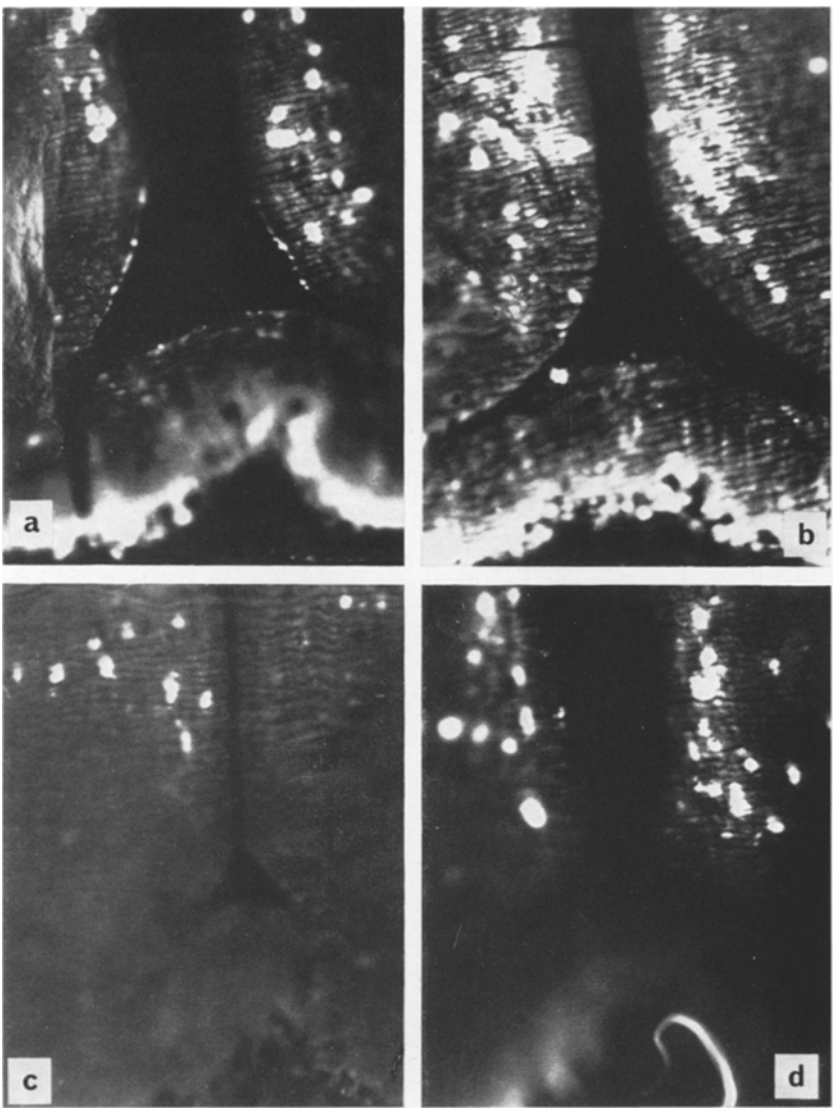

Fig. 4a—d. Hypothalamus, IIId ventricle of the rat 1 h after intraventricular administration of 0.05 mg NA in 10 µl in animals pretreated i.p. by a saline; b hash. smok. subl. 300 mg/kg; c reserpine 10 mg/kg; d reserpine + hash. smok. subl. 300 mg/kg. ×100

A decrease of catabolism by inhibition of *monoaminoxidase* is not likely: Holtzman *et al.* (1969) found that after tetrahydrocannabinol the

5HIAA in the brain is not decreased but slightly elevated. This is supported by our finding that the DOPA induced capillary green fluorescence is not increased by hashish smoke sublimate as it is after Nialamid.

There is some evidence that tetrahydrocannabinol increases the turnover and metabolism of noradrenaline in the brain: following tetrahydrocannabinol administration, the uptake of intracisternally administered ^{3}H NA is slightly increased (Schildkraut and Efron, cited by Efron, 1968). We found that the increase of green fluorescence in the hypothalamic varicosities after intraventricular administration of noradrenaline is accentuated after treatment with hashish smoke sublimate in the reserpinized and non reserpinized animal. This phenomenon may be related to a facilitation of the uptake of noradrenaline but we cannot eliminate the possibility of increased synthesis.

There is a parallelism between a) our finding that hashish increases noradrenaline in hypothalamic varicosities; b) that microinjection of noradrenaline in the hypothalamus produces hypothermia (Feldberg and Myers, 1963, 1965); c) that intrahypothalamic administration of noradrenaline induces increased food intake (Grossman, 1962); and d) that hashish smoking produces hypothermia and increase of appetite (Miras, 1965, 1969).

The prolongation of amphetamine action by cannabis derivatives is probably related to a common effect of the two drugs on serotonin, but also to an increase of uptake of noradrenaline; it is known that amphetamine lowers the noradrenaline concentration of the brain (Boyd and Meritt, 1965); the mechanism of this action may be an imipramine-like inhibition of re-uptake in the presynpatic vesicles (Carlsson *et al.*, 1966), or a facilitation of release of amines from the neurons (Fuxe and Ungerstedt, 1968); hashish, facilitating the re-uptake should prolong the amphetamine action in a competitive manner.

Increase of uptake should explain why Demethyl-Heptyl-Pyran lowered the blood pressure in cats (Dagirmanjian and Boyd, 1962), but it is difficult to explain why it potentiated the effects on blood pressure of noradrenaline and adrenaline (Dagirmanjian and Hodge, 1969).

Some other physiological actions of cannabis are also probably related to an effect on the noradrenaline varicosities especially of the reticular formation: Dimethyl-Heptyl-Pyran (0.2 mg/kg) *increases* the threshold for EEG arousal induced by electrical stimulation of the reticular formation (Boyd and Meritt, 1965, 1966); tetrahydrocannabinol (8 mg/kg) *lowers the threshold* of the arousal response elicited by direct stimulation of the reticular formation (Bircher and Mechoulam, 1968). In monkeys, tetrahydrocannabinol in doses from 4 to 8 mg/kg produces *depression* of general activity; increase of the dose to 16, 32, 64 mg/kg

produces stimulation and hallucinations (Scheckel *et al.*, 1968). In the cat, tetrahydrocannabinol at 10 mg/kg produces *relaxation*; 30 mg/kg produces insomnia for 15 h (Huerliman *et al.*, cited by Efron, 1968). If cannabis derivatives really act on the reticular formation, this action seems to be *tranquilizing in low doses and exciting in relatively high doses.*

Any similarities between the effects of hashish and LSD in man (dreamy state, illusions, hallucinations, euphoria), have different mechanisms: LSD decreases the noradrenaline and increases the serotonin levels in the brain (Freedman, 1963), but there is no cross tolerance between tetrahydrocannabinol and LSD (Isbell *et al.*, 1967; Silva *et al.*, 1968).

References

Anden, N. E., Dahlström, A., Fuxe, K., Larsson, K.: Mapping out of catecholamine and 5-hydroxytryptamine neurons innervating the telencephalon and diencephalon. Life Sci. **4**, 1275—1279 (1965).

Bertler, A., Falck, B., Owman, C., Rosengren, E.: The localisation of monoaminergic blood-brain barrier mechanisms. Pharmacol. Rev. **18**, 369—385 (1966).

Bircher, H. I., Mechoulam, R.: Pharmacological effects of two active constituents of marihuana. Arch. int. Pharmacodyn. **172**, 24—31 (1968).

Boyd, E. S., Meritt, D. A.: Effects of thiopental and a tetrahydrocannabinol derivative on arousal and recruiting in the cat. J. Pharmacol. exp. Ther. **149**, 138—145 (1965).

— — Effects of barbiturates and a tetrahydrocannabinol derivative on recovery cycles of medial lemniscus, thalamus and reticular formation in the cat. J. Pharmacol. exp. Ther. **151**, 376—384 (1966).

Carlsson, A., Falck, B., Hillarp, N. A., Torp, A.: Histochemical localisation at the cellular level of hypotalamic noradrenaline. Acta physiol. scand. **54**, 385—386 (1962).

— Fuxe, K., Hamberger, R., Lindqvist, M.: Biochemical and histochemical studies on the effects of imipramine like drugs and amphetamine on central and peripheral catecholamine neurons. Acta physiol. scand. **67**, 481—497 (1966).

Constantinidis, J., Bartholini, G., Tissot, R., Pletscher, A.: Elective Anreicherung von Dopamine im Parenchym des Rattenhirns. Helv. physiol. pharmacol. Acta **25**, 411—415 (1967).

Dagirmanjian, R., Boyd, E. S.: Some pharmacological effects of two tetrahydrocannabinols. J. Pharmacol. exp. Ther. **135**, 25—33 (1962).

— Hodge, H. C.: Are biogenic amines involved in the mecanism of action of the tetrahydrocannabinols? Agents and Actions **2**, 46—48 (1969).

Dahlström, A., Fuxe, K.: Evidence for the existence of monoamine-containing neurons in the central nervous system. Acta physiol. scand. **62**, suppl. 232 (1964).

Efron, D. H.: Marihuana: a few problems. In: Psychopharmacology. A Review of Progress 1957—1967, D. H. Efron, ed. (Public Health Service Public. No. 1836, USA), p. 1269 (1968).

Falck, B., Hillarp, N. A., Thieme, G., Torp, A.: Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. **10**, 348—354 (1962).

— Owman, C.: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Univ. (Lund.) II, 7 (1965).

Feldberg, W., Myers, R. D.: A new concept of temperature regulation by amines in the hypothalamus. Nature (Lond.) **200**, 1325 (1963).

— — Change in temperature produced by microinjections of amines into the anterior hypothalamus of cats. J. Physiol. (Lond.) **177**, 239—245 (1965).

Freedman, D. X.: Psychomimetic drugs and brain biogenic amines. Amer. J. Psychiat. **119**, 843—850 (1963).

Fuxe, K., Ungerstedt, U.: Histochemical studies on the distribution of catecholamines and 5-hydroxytryptamine after intraventricular injection. Histochemie **13**, 16—28 (1968a).

— — Histochemical studies on the effect of (+)-amphetamine, drugs of the imipramine group and tryptamine on central catecholamine and 5-hydroxytryptamine neurons after intraventricular injection of catecholamines and 5-hydroxytryptamine. Europ. J. Pharmacol. **4**, 135—144 (1968b).

Garrattini, S.: Effects of a cannabis extract on gross behaviour. In: Hashish: its Chemistry and Pharmacology, CIBA Found., Study Group No. 21, p. 70. London: J. A. Churchill 1965.

Garriott, J. C., Forney, R. B., Hughes, F. W., Richards, A. B.: Pharmacological properties of some cannabis related compounds. Arch. int. Pharmacodyn. **171**, 425—434 (1968).

— King, L. J., Forney, R. B., Hughes, F. W.: Effects of some tetrahydrocannabinols on hexobarbital sleeping time and amphetamine induced hyperactivity in mice. Life Sci. **6**, 2119—2128 (1967).

Goodman, L. S., Gilman, A.: Cannabis (Marihuana). In: The Pharmacological basis of Therapeutis, 3th Ed., p. 299. New York: MacMillan 1965.

Grossman, S. P.: Amer. J. Physiol. **202**, 872 (1962); cited by S. R. Sommer, D. Novin and M. Levin,: Food and water intake after intrahypothalamic injections of carbachol in the rabbit. Science **156**, 883—884 (1967).

Hayden, J. F., Johnson, L. R., Maickel, M. P.: Construction and implantation of permanent cannula for making injections into the lateral ventricle of rat brain. Life Sci. **5**, 1509—1515 (1966).

Holtzmann, D., Lovell, R. A., Jaffe, J. H., Freedmann, D. X.: 1-delta 9-tetrahydrocannabinol: neurochemical and behavioral effets in the mouse. Science **163**, 1464—1467 (1969).

Isbell, H., Gorodetzsky, C. W., Jasinski, D., Claussen, U., Spulak, F. v., Korte, F.: Effects of (—) delta-9-trans-tetrahydrocannabinol in man. Psychopharmacologia (Berl.) **11**, 184—188 (1967).

Joachimoglou, G.: Natural and smoked hashish. In: Hashish: its Chemistry and Pharmacology. Ciba Found., Study Group No. 21, p. 2. London: J. A. Churchill 1965.

Kubena, R. K.., Barry, H.: Interactions of delta 1-tetrahydrocannabinols with barbiturates and methamphetamine. J. Pharmacol. exp. Ther. **173**, 94—100 (1970).

Miras, C. J.: Some aspects of cannabis actions. In: Hashish: its Chemistry and Pharmacology, Ciba Found., Study Group No. 21, p. 37. London: J. A. Churchill 1965.

— Experience with chronic hashish smokers. In: Drugs and Yuth, p. 191. New York: Ch. C. Thomas 1969.

— Kephalas, T. A., Papadakis, D. P.: The effect of hashish extract on the norepinephrine in rabbit brain. Narcotic's Bull. (in press).

Robertis, E., de, Iraldi, A. P., de, Lores-Arnaiz, G. R., de, Zieher, L. M.: Synaptic vesicles from the rat hypothalamus. Isolation and norepinephrine content. Life Sci. **4**, 193—201 (1965).

Scheckel, C. L., Boff, E., Dahlen, P., Smart, T.: Behavioral effects in monkeys of racemate of two biologically active marihuana constituents. Science **160**, 1467—1469 (1968).

Silva, M. T., Carlini, E. A., Claussen, U., Korte, F.: Lack of cross-tolerance in rats among (—) delta 9-trans-tetrahydrocannabinol (delta-9-THC), cannabis extract, mescaline, and lysergic acid diethylamid (LSD-25). Psychopharmacologia (Berl.) **13**, 332—340 (1968).

Smith, C. B.: Effects of D-amphetamine upon brain amine content and locomotor activity of mice. J. Pharmacol. exp. Ther. **147**, 96—102 (1965).

Vieira, F. J. A., Aguiar, M. B., Alencar, J. W., Seabra, A. P., Tursch, B. M., Leclercq, J.: Effects of the organic layer of hashish smoke extract and preliminary results of its chemical analysis. Psychopharmacologia (Berl.) **10**, 361—362 (1967).

Watt, I. M.: Drug dependence of hashish type. In: Hashish: its chemistry and pharmacology. Ciba Found., Study Group No. 21, p. 54. London: J. A. Churchill 1965.

Dr. J. Constantinidis
University of Geneva
Department of Psychiatry
Bel-Air, 1225 Geneva
Switzerland