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TOLERANCE TO THE CARDIOVASCULAR EFFECTS OF Δ^9-TETRAHYDROCANNABINOL IN THE RAT

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1 Daily intraperitoneal injections of Δ^9-tetrahydrocannabinol (Δ^9-THC, 10 mg/kg) resulted in tolerance to the effects of the cannabinoid on body weight and body temperature within 1–2 weeks of treatment.

2 Tolerance failed to develop to the suppression of spontaneous motor activity produced by Δ^9-THC during 28 days of treatment with the cannabinoid (10 mg/kg, i.p. per day).

3 Following treatment with vehicle for 28 days, intravenous administration of Δ^9-THC in anaesthetized rats produced a transient pressor response followed by a sustained hypotension and bradycardia.

4 Tolerance to the hypotensive and negative chronotropic responses to intravenous Δ^9-THC was readily apparent in animals which had received daily intraperitoneal injections of Δ^9-THC (10 mg/kg) for 28 days.

5 Tolerance failed to develop to the pressor actions of intravenous Δ^9-THC after 28 days of pretreatment.

6 There was no difference in the pressor response to intravenous noradrenaline in vehicle-treated animals (1.0 ml/kg, i.p., per day for 28 days) and Δ^9-THC-treated animals (10 mg/kg, i.p., per day for 28 days).

Introduction

Tolerance to the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) or extracts of marihuana has been demonstrated by several investigators in a variety of experimental situations in laboratory animals. McMillan, Harris, Frankenheim & Kennedy (1970) demonstrated a reduction in the inhibitory effect of Δ^9-THC on key pecking behaviour in the pigeon. Others have shown tolerance to the effects of Δ^9-THC on bar pressing behaviour in rats and pigeons (Ford & McMillan, 1971), on overt behaviour (static ataxia) in the dog (Dewey, Jenkins, O'Rourke & Harris, 1972), on the conditioned avoidance response (Bailey, Pradhan & Ghosh, 1971), on body weight (Graham & Li, 1973), on locomotor activity (Davis, Moreton, King & Pace, 1972), on body temperature in the rat (Sofia, 1972), and on locomotor activity and body temperature in the neonatal chick (Abel, 1972).

Studies designed to determine whether tolerance develops to the cardiovascular actions of Δ^9-THC have been contradictory and inconclusive. Graham & Li (1973) demonstrated a reduction in the hypotensive and negative chronotropic effects of an intravenously administered extract of cannabis in rats following 14 days of pretreatment with the extract. Also, Nahas, Schwartz, Adamec & Manger (1973) presented evidence for the rapid development of tolerance to the hypotensive effects of Δ^9-THC in spontaneously hypertensive rats. However, studies by Birmingham (1973), Williams, Ng, Lamprecht, Roth & Kopin (1973) and Lewis, Brown & Forney (1974), failed to demonstrate tolerance to the hypotensive actions of Δ^9-THC in either hypertensive (spontaneous or adrenal-regeneration) or normotensive animals following pretreatment periods ranging from 7–14 days.

Recent studies in this laboratory indicate that intraarterially administered Δ^9-THC produces vasoconstriction in the perfused hind-quarters of the rat (Adams, Dewey & Harris, 1974). Furthermore, it was observed that intravenously administered Δ^9-THC produced transient, dose-related pressor responses that were followed by the characteristic hypotensive and negative chronotropic effects. Therefore, this study was undertaken to determine if pretreatment with Δ^9-THC produces tolerance to the pressor, depressor, and negative chronotropic effects of intravenously administered Δ^9-THC in normotensive rats. In addition, pressor responses to noradrenaline were evaluated in the light of recent data suggesting

that Δ^9-THC might act as an adrenergic neurone blocking agent (Graham, Lewis & Li, 1974). Effects of Δ^9-THC treatment on body weight, spontaneous motor activity, and body temperature, were examined during the course of the treatment schedule to assess tolerance development.

Methods

Male, Sprague-Dawley rats (Flow Laboratories, Dublin, Virginia, U.S.A.) with initial body weights ranging from 245–270 g were used for this study. Animals were housed in individual cages in temperature-controlled facilities (21–22°C) with an alternating 12 h dark-light cycle. Animals had access to standard laboratory chow and drinking water *ad libitum*.

Treatment schedules

Sixteen rats were randomly assigned to two groups designated as vehicle-treated and Δ^9-THC-treated, respectively. Δ^9-THC-treated animals received daily, intraperitoneal injections of 10 mg/kg Δ^9-THC (solubilized in an ethanol: Emulphor EL-620: saline vehicle) for 28 days. Vehicle-treated animals received daily injections of equivalent volumes, on a weight basis, of vehicle alone for 28 days. Body weight was recorded daily at the time of injection with all injections made between 11 h 00 min and 13 h 15 min. During the course of the treatment schedule, two animals from the Δ^9-THC group and one animal from the vehicle-control group were killed because of the appearance of symptoms indicative of a respiratory tract infection.

Measurement of body temperature

Body temperature was measured with a rectal probe and a telethermometer (Model 43-T3, Yellow Springs Instrument Company, Yellow Springs, Ohio, U.S.A.). The hypothermic response to Δ^9-THC was evaluated on days 1, 5, 12, 20 and 28 of the treatment schedule by recording the temperature of all animals, before and 70 min after the administration of either Δ^9-THC or the vehicle. Data were expressed as the change (°C) from the pre-injection value.

Measurement of spontaneous motor activity

Spontaneous motor activity was measured on a Lafayette Activity Platform (Lafayette Instrument Company, Lafayette, Indiana, U.S.A.) which records all types of movement. Total activity (counts) was recorded for 10 min periods during each testing session. Spontaneous motor activity was evaluated 60 min after injection of either Δ^9-THC or vehicle on days 1, 8, 15, 20 and 28 of the treatment schedule.

Measurement of blood pressure and heart rate

On the day following the final injection of either Δ^9-THC or vehicle, all animals were anaesthetized with urethane (1.2 g/kg, i.p.) and prepared for intravenous drug administration and measurement of blood pressure and heart rate. After insertion of a tracheal cannula, a polyethylene cannula (PE-20) was placed in the right external jugular vein for drug administration. The left carotid artery was cannulated (PE-90) and blood pressure monitored by a Statham p-23A transducer and recorded on a Grass Model 7B polygraph. The mean arterial pressure was calculated as follows: mean arterial pressure (mmHg) = diastolic pressure + 1/3 (systolic pressure − diastolic pressure). Heart rate was determined from the blood pressure tracing. Drugs were administered in the jugular cannula in 0.1 ml volumes and flushed with 0.2 ml of saline (0.9% w/v NaCl solution).

After a 5 min equilibration period, each animal received noradrenaline 0.3 μg/kg and the pressor response was determined. After blood pressure returned to the control value, each animal received an injection of the vehicle used for Δ^9-THC and blood pressure was recorded during the first minute and 3, 5 and 10 min after vehicle administration. Heart rate was recorded 3, 5 and 10 min after vehicle administration. Finally each animal received Δ^9-THC 1.0 mg/kg, blood pressure was recorded during the first minute after injection and both blood pressure and heart rate were recorded at 3, 5, 10, 20, 30 and 40 min after administration of Δ^9-THC.

Drugs

Δ^9-THC was prepared as a solution (100 mg/ml) in a 1:1 mixture of absolute ethanol and a polyoxyethylated vegetable oil (Emulphor EL-620; GAF Corporation, New York, New York, U.S.A.). This stock solution was diluted with saline to yield appropriate concentrations such that 0.1 ml would deliver a dose of 1.0 mg/kg. Similar concentrations of vehicle were employed as control injections. Noradrenaline bitartrate (Levophed; Winthrop Laboratories, New York, New York, U.S.A.) was diluted with saline such that 0.1 ml would provide a dose of 0.3 μg/kg of the free base.

Statistics

Statistical tests employed in the analysis of data include Student's t test, paired t test, and a multivariate profile analysis described by Morrison (1967). Values of $P < 0.05$ were considered significant.

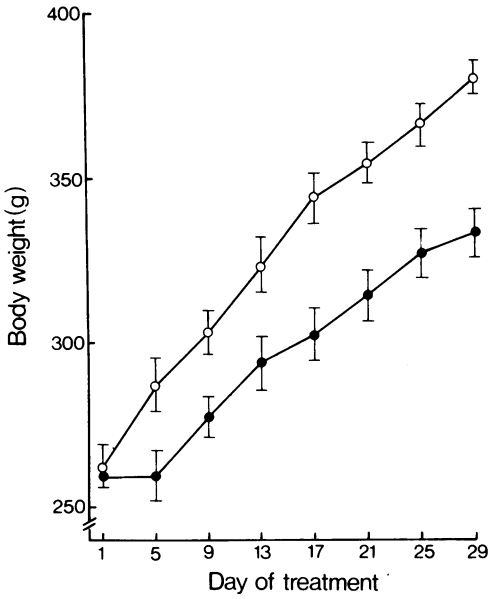

Figure 1 Effects of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) on body weight. Rats treated daily with vehicle 1 mg/kg, i.p. (○); rats treated daily with Δ⁹-THC, 10 mg/kg, i.p. (●). Each point represents the mean of results from 6–8 animals. Vertical lines represent s.e. mean. Lines between days 1 and 5 are not parallel ($P < 0.002$); while segments between days 5 and 29 are parallel ($P > 0.14$).

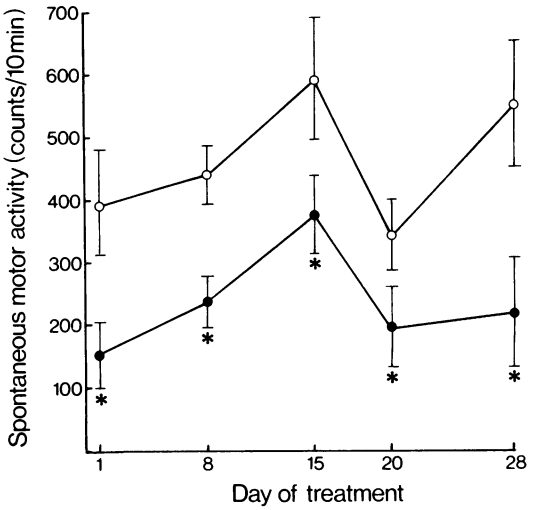

Figure 2 Effects of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) on spontaneous motor activity. Rats treated daily with vehicle, 1 ml/kg, i.p. (○); rats treated daily with Δ⁹-THC, 10 mg/kg, i.p. (●). Each point represents the mean of results from 6–8 animals. Vertical lines represent s.e. mean.
* Significant difference between groups (minimum $P < 0.05$).

Results

Effects of Δ⁹-THC on body weight

The effects of daily injections of Δ⁹-THC on body weight are illustrated in Figure 1. Body weights were recorded daily but for clarity and statistical evaluation only the data shown were employed. Vehicle-treated animals exhibited a normal growth curve and gained weight throughout the course of the experiment. Δ⁹-THC-treated animals failed to gain weight during the first five days of treatment and weighed significantly less than vehicle-treated animals on day 5. However, from day 5 to the end of the treatment period, Δ⁹-THC-treated animals gained weight at the same rate as vehicle-treated animals, indicating that tolerance to the depressant effects of Δ⁹-THC on body weight had developed.

Effects of Δ⁹-THC on spontaneous motor activity

Figure 2 clearly illustrates that under the conditions of this experiment, tolerance did not develop to the effects of Δ⁹-THC on spontaneous motor activity. On all days in which effects of Δ⁹-THC on spontaneous motor activity were examined, there were significant differences in activity between control and Δ⁹-THC-treated rats.

Effects of Δ⁹-THC on body temperature

Initial treatment (day 1) with Δ⁹-THC (10 mg/kg, i.p.) produced a marked decrease in body temperature as indicated in Figure 3. Significant differences in the body temperatures of vehicle- and Δ⁹-treated animals were still apparent on days 5 and 8 of treatment, although the magnitude of the hypothermic response was significantly reduced when compared to day 1. On day 12, there was no significant hypothermic response in the Δ⁹-THC-treated rats but a slight hypothermia was noted on day 15. However, on days 20 and 28, there were no significant differences in body temperature responses to vehicle or Δ⁹-THC, indicating tolerance to the hypothermic actions of Δ⁹-THC.

Effects of Δ⁹-THC-pretreatment on the blood pressure responses to intravenously administered noradrenaline and Δ⁹-THC

Initial mean arterial pressures in the anaesthetized animals were 79.0 and 78.5 mmHg, respectively, for

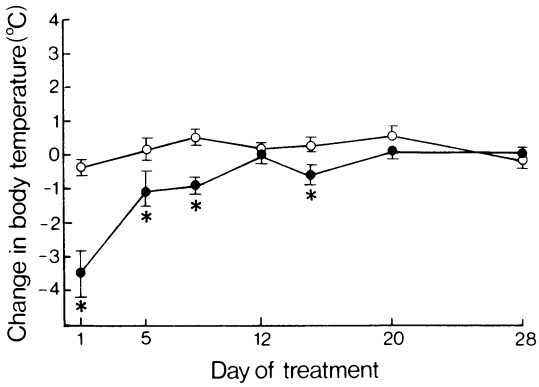

Figure 3 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on body temperature. Rats treated daily with vehicle, 1 ml/kg, i.p. (○); rats treated daily with Δ^9-THC, 10 mg/kg, i.p. (●). Each point represents the mean of results from 6–8 animals. Vertical lines represent s.e. mean.
* Significant difference between groups (minimum $P < 0.05$).

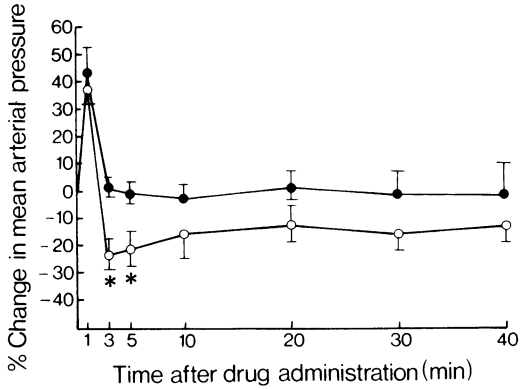

Figure 4 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) pretreatment on the blood pressure response to intravenously administered Δ^9-THC (1.0 mg/kg). Rats receiving 28 daily injections of vehicle, 1 ml/kg, i.p. (○); rats treated daily with Δ^9-THC, 10 mg/kg, i.p. (●). Each point represents the mean of results from 6 animals. Vertical lines represent s.e. mean.
* Significant difference between groups (minimum $P < 0.05$).

vehicle-pretreated and Δ^9-THC-pretreated animals. There was no significant difference in the maximum pressor response to noradrenaline (0.3 μg/kg, i.v.) in vehicle-pretreated and Δ^9-THC pretreated rats (49.2 as compared with 40.7 mmHg).

In animals pretreated for 28 days with vehicle, the intravenous administration of Δ^9-THC produced the biphasic effect on blood pressure (Figure 4) previously observed in anaesthetized rats (Adams *et al.*, 1974). Following a transient increase in pressure, there was a more sustained hypotensive effect. In the Δ^9-THC-pretreated animals, intravenous Δ^9-THC produced a pressor response equivalent to that seen in the vehicle-pretreated group. However, the hypotensive phase of the blood pressure response was absent in the Δ^9-THC pretreated animals indicating development of tolerance to this action of Δ^9-THC. Intravenous injections of vehicle produced small, transient increases in pressure which were not dose-related and were possibly due to the volume of injection. This vehicle pressor response was subtracted from the drug-induced pressor response in each animal. The vehicle did not have hypotensive activity.

Effects of Δ^9-THC pretreatment on the heart rate response to intravenously administered Δ^9-THC

Initial heart rates did not differ significantly in vehicle-pretreated and Δ^9-THC-pretreated animals (268.3 as compared with 304.0). The negative chronotropic effect of acutely administered Δ^9-THC was reduced significantly by the 28 days pretreatment with Δ^9-

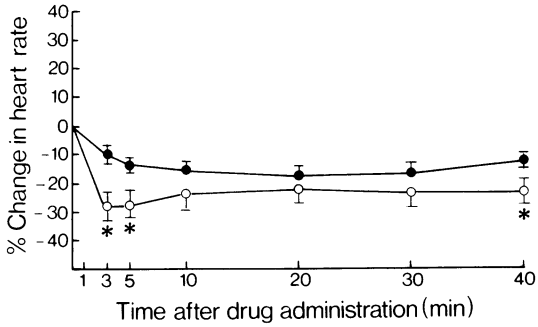

Figure 5 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) pretreatment on the heart rate response to intravenously administered Δ^9-THC (1.0 mg/kg). Rats receiving 28 daily injections of vehicle, 1 ml/kg, i.p. (○); rats treated daily with Δ^9-THC, 10 mg/kg, i.p. (●). Each point represents the mean of results from 6 animals. Vertical lines represent s.e. mean.
* Significant difference between groups (minimum $P < 0.05$).

THC as shown in Figure 5. However, in contrast to the complete absence of the hypotensive response, Δ^9-THC did produce bradycardia (10–15%) in these animals. Intravenous administration of the vehicle failed to alter heart rate in either group of animals.

Discussion

These studies confirm that tolerance develops to the effects of Δ^9-THC on body weight and body

temperature in the rat. Food consumption was not measured in this study; however, it was assumed that the failure of Δ^9-THC-treated animals (10 mg/kg per day, i.p.) to gain weight during the first 4 days of treatment could be attributed to the anorexic effect reported by a number of investigators (Manning, McDonough, Jr, Elsmore, Saller & Sodetz, 1971; Jarbe & Henriksson, 1973; Sofia & Barry, 1974). Tolerance to the effects of Δ^9-THC on body weight and body temperature developed within 5 days during the daily treatment regimen.

Rats failed to exhibit tolerance to the depressant effects of Δ^9-THC 10 mg/kg on spontaneous motor activity during 28 days of treatment. This is in contrast to the report by Davis *et al.* (1972) who demonstrated tolerance to the suppression of spontaneous motor activity after 11 days of daily intraperitoneal injections of 25 mg/kg of Δ^9-THC. In addition to the dosage differences, there are other variations in the experimental procedures (drug vehicles, recording devices, duration of recording sessions, etc.) which rule out direct comparisons between this study and that of Davis and co-workers.

Following 28 days of pretreatment with Δ^9-THC, there was clearly tolerance to the hypotensive and negative chronotropic effects of intravenously administered Δ^9-THC in anaesthetized rats. The characteristic hypotensive response to Δ^9-THC was abolished by the Δ^9-THC pretreatment regimen and the reduction in heart rate was significantly less (40–50%) than in vehicle-pretreated animals. These data are consistent with the previous report by Graham & Li (1973) who demonstrated tolerance in rats, to the hypotensive and negative chronotropic effects of an intravenously administered extract of cannabis following 14 days of treatment with that extract. However, Lewis *et al.* (1974) found no evidence of tolerance to the hypotensive action of Δ^9-THC in spontaneously hypertensive rats after 9 days of treatment with up to 10 mg/kg per day of Δ^9-THC.

Recently, Graham *et al.* (1974) presented evidence that Δ^9-THC could interfere with the release of noradrenaline during transmural stimulation of the rat isolated vas deferens. These investigators concluded that Δ^9-THC may have adrenergic neurone blocking properties. Because of the well-documented ability of pretreatment with adrenergic neurone blocking agents to elicit supersensitivity to exogenous noradrenaline (Burn & Rand, 1958), the pressor actions of intravenous noradrenaline were examined in rats pretreated with Δ^9-THC or vehicle for 28 days. There was, however, no indication of supersensitivity to noradrenaline in Δ^9-THC pretreated animals as compared to vehicle pretreated controls.

As previously reported (Adams *et al.*, 1974), intravenously administered Δ^9-THC elicits a biphasic blood pressure response in the anaesthetized rat. This response consists of a transient pressor phase followed by a more sustained reduction in blood pressure which is accompanied by bradycardia. Furthermore, the intraarterial injection of Δ^9-THC into the perfused hindquarters of the rat produces dose-related constrictor responses. This suggests that the initial pressor phase following intravenous administration may be the result of an increase in peripheral resistance. The current study clearly shows that tolerance to the pressor effect of Δ^9-THC did not develop during this treatment regimen. As previously stated, Δ^9-THC pretreated animals did exhibit a reduction in heart rate (10–15%) following acute administration of Δ^9-THC in the absence of any hypotensive response. This would indicate that an increase in stroke volume and/or an increase in peripheral resistance is acting to offset the reduction in blood pressure that might result from the bradycardia.

In conclusion, these studies clearly demonstrate that tolerance develops to the hypotensive and negative chronotropic effects of Δ^9-THC in rats previously shown to be tolerant to the body weight and hypothermic effects of the compound. However, there was no tolerance to the transient pressor activity of Δ^9-THC. The hypothermia, reduced growth rate, hypotension, and bradycardia produced by Δ^9-THC are probably manifestations of central nervous system actions (Hardman, Domino & Seevers, 1957; Manning *et al.*, 1971; Lomax, 1971; Hardman, Domino & Seevers, 1971; Cavero, Buckley & Jandhyala, 1973a; Cavero, Solomon, Buckley & Jandhyala, 1973b) while previous studies in this laboratory suggest that the pressor response is a peripheral vascular effect. Thus, it would appear that while tolerance to actions of Δ^9-THC in the central nervous system can readily be demonstrated, there is no tolerance to what appears to be a peripheral effect of Δ^9-THC. This is analagous to the absence of tolerance to the effects of morphine on intestinal smooth muscle at a time when tolerance to the CNS effects of the narcotic can easily be demonstrated.

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References

ABEL, E.L. (1972). Studies of tolerance to 1-Δ^9-tetrahydrocannabinol (Δ^9-THC) in neonatal chicks. *Fedn Proc.*, **31**, 505 Abstract.

ADAMS, M.D., DEWEY, W.L. & HARRIS, L.S. (1974). Cardiovascular effects of Δ^8- and Δ^9-tetrahydrocannabinol in rats. *Pharmacologist*, **16**, 260 Abstract.

BAILEY, P.T., PRADHAN, S.N. & GHOSH, B. (1971). Some behavioural effects of Δ^9-tetrahydrocannabinol (Δ^9-THC). *Fedn Proc.*, **30**, 279 Abstract.

BIRMINGHAM, M.K. (1973). Reduction by Δ^9-tetrahydrocannabinol in the blood pressure of hypertensive rats bearing regenerated adrenal glands. *Br. J. Pharmac.*, **48**, 169–171.

BURN, J.H. & RAND, M.J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol.*, **144**, 314–336.

CAVERO, I., BUCKLEY, J.P. & JANDHYALA, B.S. (1973a). Hemodynamic and myocardial effects of (–)-Δ^9-trans-tetrahydrocannabinol in anesthetized dogs. *Eur. J. Pharmac.*, **24**, 243–251.

CAVERO, I., SOLOMON, T., BUCKLEY, J.P. & JANDHYALA, B.S. (1973b). Studies on the bradycardia induced by (–)-Δ^9-trans-tetrahydrocannabinol in anesthetized dogs. *Eur. J. Pharmac.*, **22**, 263–269.

DAVIS, W.M., MORETON, J.E., KING, W.T. & PACE, H.B. (1972). Maihuana and locomotor activity: biphasic effect and tolerance development. *Res. Commun. Chem. Path. Pharmac.*, **3**, 29–35.

DEWEY, W.L., JENKINS, J., O'ROURKE, T. & HARRIS, L.S. (1972). The effects of chronic administration of trans-Δ^9-tetrahydrocannabinol on behavior and the cardiovascular system of dogs. *Arch. int. Pharmacodyn. Ther.*, **198**, 118–131.

FORD, R.D. & McMILLAN, D.E. (1971). Behavioural tolerance and cross tolerance to 1-Δ^8-tetrahydrocannabinol (Δ^8-THC) and 1-Δ^9-tetrahydrocannabinol (Δ^9-THC) in pigeons and rats. *Fedn. Proc.*, **30**, 279 Abstract.

GRAHAM, J.D.P. & LI, D.M.F. (1973). Cardiovascular and respiratory effects of cannabis in cat and rat. *Br. J. Pharmac.*, **49**, 1–10.

GRAHAM, J.D.P., LEWIS, M.J. & LI, D.M.F. (1974). The effect of Δ^1-tetrahydrocannabinol on the release of [^{3}H]-(1)-noradrenaline from the isolated vas deferens of the rat. *Br. J. Pharmac.*, **52**, 233–236.

HARDMAN, H.F., DOMINO, E.F. & SEEVERS, M.H. (1957). The chemistry and pharmacology of certain compounds affecting the central nervous system of animals and man. *Clearinghouse for Federal Scientific and Technical Information*, AD 707–699, pp. 1–53.

HARDMAN, H.F., DOMINO, E.F. & SEEVERS, M.H. (1971). General pharmacological actions of some synthetic tetrahydrocannabinol derivatives. *Pharmacol. Rev.*, **23**, 295–315.

JARBE, T.U.C. & HENRIKSSON, B.G. (1973). Acute effects of two tetrahydrocannabinols (Δ^9-THC and Δ^8-THC) on water intake in water deprived rats: implications for behavioral studies on marijuana compounds. *Psychopharmacologia*, **30**, 315–322.

LEWIS, S.C., BROWN, D.J. & FORNEY, R.B. (1974). Absence of tolerance to the hypotensive effects of delta-9-tetrahydrocannabinol in hypertensive rats. *Toxicol. Appl. Pharmac.*, **28**, 7 Abstract.

LOMAX, P. (1971). Acute tolerance to the hypothermic effect of marihuana in the rat. *Res. Commun. Chem. Path. Pharmac.*, **2**, 159–167.

McMILLAN, D.E., HARRIS, L.S., FRANKENHEIM, J.M. & KENNEDY, J.S. (1970). I-Δ^9-trans-tetrahydrocannabinol in pigeons: tolerance to the behavioral effects. *Science*, **169**, 501–503.

MANNING, F.J., McDONOUGH, J.H., Jr., ELSMORE, T.F., SALLER, C. & SODETZ, F.J. (1971). Inhibition of normal growth by chronic administration of Δ^9-tetrahydrocannabinol. *Science*, **174**, 424–426.

MORRISON, D.F. (1967). *Multivariate statistical methods.* New York, N.Y.: McGraw-Hill Publishing Co.

NAHAS, G.G., SCHWARTZ, I.W., ADAMEC, J. & MANGER, W.M. (1973). Tolerance to delta-9-tetrahydrocannabinol in the spontaneously hypertensive rat. *Proc. Soc. exp. Biol. Med.*, **142**, 58–60.

SOFIA, R.D. (1972). A paradoxical effect for Δ^1-tetrahydrocannabinol on rectal temperature in rats. *Res. Commun.Chem. Path. Pharmac.*, **4**, 281–288.

SOFIA, R.D. & BARRY, H. (1974). Acute and chronic effects of Δ^9-tetrahydrocannabinol on food intake by rats. *Psychopharmacologia*, **39**, 213–222.

WILLIAMS, R.B., NG, L.K.Y., LAMPRECHT, F., ROTH, K. & KOPIN, I.J. (1973). Δ^9-tetrahydrocannabinol: a hypotensive effect in rats. *Psychopharmacologia*, **28**, 269–274.

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