The content of (−)Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) does not explain all biological activity of some Brazilian marihuana samples

Carlini, Santos & others (1970) reported that the biological activity (Gayer test in rabbits) of 5 extracts obtained from marihuana seized by the German Police corresponded to the content of Δ^9-THC present in the material; thus, extracts containing respectively, 27·6, 26·5, 9·8, 4·6 and 0·09% of Δ^9-THC, when assayed on rabbits, showed respectively 46·2, 34·9, 7·8, 1·8 and 0·38% of the activity of that of pure Δ^9-THC used as standard. It was then suggested that the Gayer test could be useful for giving an approximate measure of Δ^9-THC content in cannabis extracts.

We have now made similar experiments with flowering tops of 2 samples of Brazilian marihuana. Chemical determinations of the samples by gas chromatography were carried out by several laboratories (Table 1). The Δ^9-THC content in each sample varied according to the laboratory, although sample B always possessed nearly twice as much Δ^9-THC as sample A. The plants were then extracted in light petroleum as recommended by Carlini & Kramer (1965); marihuanas A and B yielded, respectively, 12·06 and 14·56% of resin. Suspensions made up of these resinous materials and suspensions of pure Δ^9-THC in saline plus Tween-80 (Carlini & Kramer, 1965) were assayed on rabbits (Gayer test), rats (climbing rope test) and mice (catatonia and spontaneous motor activity tests), according to Carlini, Silva & others (1967, 1970). The results, summarized in Table 2, show that sample B was about twice as active as sample A as would be expected from their difference in Δ^9-THC content; however, when these results are compared to those obtained with pure Δ^9-THC the extracts are 3 to 5 times more active than expected.

Marihuanas A and B and Δ^9-THC were also administered to men by smoking; again B was twice as active as A, and each was about 3 times more active than would

Table 1. *Gas chromatography analysis of Brazilian marihuanas A and B by different laboratories.*

	Percentage of constituents in marihuana A and B							
	Δ^8-THC		Δ^9-THC		Cannabinol		Cannabidiol	
Laboratories	A	B	A	B	A	B	A	B
NIMH	0·1	0·1	1·80	2·50	1·7	1·9	0·2	0·2
RTI	—	—	0·82	2·02	1·56	2·12	0·06	0·16
UM	—	—	0·94	1·80	1·9	3·3	0·089	0·12
DFUNH	—	—	0·70	1·35	—	—	—	—

NIMH—National Institute of Mental Health (USA); RTI—Research Triangle Institute from North Carolina (USA); UM—Department of Pharmacology of the University of Mississippi (USA); DFUNH—Department of Pharmacology, University of Nijmegen, The Netherlands. We are very grateful to the colleagues who have done the assays.

Table 2. *Effects of marihuana extracts A, B and Δ^9-THC on rabbits, mice and rats.*

Drug	Gayer test (mg/kg ± s.d.) (rabbits)	Catatonia ED50 (mg/kg ± s.e.) (mice)	Motor activity ED50 (mg/kg ± s.e.) (mice)	Climbing rope ED50 (mg/kg ± s.e.) (rats)
Δ^9-THC	0·10 ± 0·03	34·1 ± 10·4	18·7 ± 7·0	11·3 ± 3·8
Extract A	0·23 ± 0·06	52·5 ± 36·1	85·2 ± 79·5	26·3 ± 13·8
Extract B	0·16 ± 0·06	33·9 ± 10·0	37·3 ± 26·8	13·5 ± 5·4

Table 3. *Correlation between chemical composition and pharmacological activity of semi-purified extracts of* Cannabis sativa.

Extract	Percentage of constituents		Ratio Δ^9-THC: cannabidiol	Ratio Δ^9-THC: cannabinol	% activity compared to Δ^9-THC (100%) in Gayer test (rabbits)	Motor activity (mice)	Catatonia (mice)	Climbing rope (rats)
A (Brazilian)	Δ^9-THC	8·8	9·6	0·6	46·0	24·0	79·0	46·0
	Δ^8-THC	0·8						
	Cannabidiol	0·9						
	Cannabinol	14·2						
B (Brazilian)	Δ^9-THC	13·2	12·1	0·7	70·0	50·0	108·0	92·0
	Δ^8-THC	0·6						
	Cannabidiol	1·0						
	Cannabinol	16·7						
1.1 TH (German)	Δ^9-THC	27·6	2·2	3·0	46·2	40·5	48·9	—
	Δ^8-THC	—						
	Cannabidiol	12·5						
	Cannabinol	9·2						

be expected from their Δ^9-THC content (Karniol & Carlini, submitted for publication).

From the yield of resin and chemical assay (Table 1), it was possible to derive the chemical compositions of the extracts and to compare them with those from extracts of the German marihuanas. Table 3, shows the German extract '1·1 TH' is, respectively, 4 and 2 times richer in Δ^9-THC than Brazilian samples A and B; however, its biological activity is nearly equal to that of sample A and half of sample B. In man a similar result was obtained; 250 mg of marihuana B smoked by the volunters, which from the chemical composition contained about 6 mg of Δ^9-THC, induced perceptual and psychological changes comparable to that obtained by smoking 20 mg of pure Δ^9-THC.

The Brazilian and German extracts differ markedly in their cannabidiol and cannabinol contents. Brazilian samples are much richer in cannabinol and poorer in cannabidiol; this can be best seen in the ratios Δ^9-THC: cannabidiol and Δ^9-THC: cannabinol (Table 3). Several possibilities are suggested to explain our results. First, it is possible that a major portion of Δ^9-THC in Brazilian samples was converted to cannabinol (Levine, 1944; Lerner, 1969; Doorenbos, Fetterman & others, 1971), probably due either to deterioration with time or to transport conditions from Brazil to other countries (WHO Scientific Group Report, 1971). This would explain the high content of cannabinol in samples A and B. Second, it could be that the large amount of cannabidiol in the German sample was inhibiting the activity of Δ^9-THC. In this respect, Carlini & others (1970) have suggested that cannabidiol was able to block the effects of Δ^9-THC on spontaneous motor activity of mice. Finally, it should also be considered that cannabinol present in large quantities in Brazilian marihuanas could be potentiating the activity of Δ^9-THC. The possible interference of cannabidiol and cannabinol on the effects of Δ^9-THC has been suggested before (Isbell, 1971).

Setor de Psicofarmacologia,
Departamento de Bioquimica e Farmacologia,
Escola Paulista de Medicina, Rua Botucatu,
862–04023 Sao Paulo, Brazil.

I. G. KARNIOL
E. A. CARLINI

May 4, 1972

REFERENCES

CARLINI, E. A. & KRAMER, C. (1965). *Psychopharmacologia (Berl.)*, **7**, 175–181.

CARLINI, E. A., SANTOS, M., CLAUSSEN, V., BIENIEK, D. & KORTE, F. (1970). *Psychopharmacologia (Berl.)*, **18**, 83–93.

CARLINI, E. A., SILVA, M. T. A., CESARE, L. C. & ENDO, R. M. (1967). *Med. Pharmac. Exp.*, **17**, 534–542.

DOORENBOS, N. J., FETTERMAN, P. S., QUIMBY, M. W. & TURNER, C. E. (1971). *Ann. N.Y. Acad. Sci.*, **191**, 3–14.

ISBELL, H. (1971). *Pharmac. rev.*, **23**, 337–338.

LERNER, P. (1969). *Bull. Narcot.*, **21**, 39–42.

LEVINE, J. (1944). *J. Amer. chem. Soc.*, **66**, 1968.

WHO SCIENTIFIC GROUP REPORT (1971). *Wld. Hlth. Org. techn. Rep. Ser.*, no. 478, 1–46.

Changes in body core and skin temperature following intracerebroventricular injection of substances in the conscious rat: interpretation of data

During experiments to investigate the role of proposed transmitter substances in regulating body temperature in the conscious rat, it became apparent that many such substances injected intracerebroventricularly (i.c.) caused a fall in core temperature, accompanied by a rise in skin temperature. Accordingly, it was decided to extend the initial study to include a wider range of naturally occurring and synthetic substances, to see if any correlation could be established between the known receptor activity of these drugs and the response obtained.

A permanent cannula was implanted in the left lateral ventricle of male hooded rats by the method of Hayden, Johnson & Maickel (1966). All drugs were dissolved in 0·9% w/v sterile saline and injected in a volume of 10 μl/rat. Oesophageal and skin temperature (base of tail) were measured using probes connected to an electric thermometer. The temperature changes quoted relate to mean differences from the temperature immediately before injection.

Drugs that are adrenoceptor agonists, or mediate their effects at or through dopamine or 5-hydroxytryptamine (5-HT) receptors, caused a significant fall in core temperature ($P < 0{\cdot}05$) and a significant rise in skin temperature ($P < 0{\cdot}05$) following i.c. injection (Table 1). The peak responses of core temperatures usually occurred after 10 min with all drugs except oxymetazoline, ergotamine and apomorphine, for which the peak fall occurred after 25 min. Peak rises in skin temperature invariably occurred after 5 min, with the exception of the latter three substances to which, the peak response occurred after 15–25 min. Acetylcholine and vasopressin also caused hypothermia after i.c. injection which was preceded by a rise in skin temperature. Papaverine, salbutamol and isoprenaline did not modify core temperature following i.c. injection. However, salbutamol and papaverine, but not isoprenaline, lowered skin temperature which for salbutamol was a significant effect ($P < 0{\cdot}05$).

The fall in core temperature is secondary to the rise in skin temperature. The latter effect results from vasodilation of peripheral vessels and can only be due to inhibition of sympathetic tone. Thus peripheral vasodilation is the common mediating mechanism whereby loss of core heat is effected. Centrally however, several mechanisms could control such peripheral changes e.g. (i) Activation of thermoregulatory centres; (ii) activation of sympathoinhibitory centres; (iii) alterations in local cerebral blood flow.

Firstly, in accord with Feldberg & Myers (1964), the drugs could all be acting on neurons mediating thermoregulatory heat dissipating mechanisms. It is remarkable, if this is the case, that such a variety of drugs all affect body temperature in a qualitatively similar fashion. Folkow, Johansson & Oberg (1959), Lofving (1961) and Folkow, Langston & others (1964) have shown that electrical stimulation of the anterior hypothalamic region results in generalized peripheral sympathetic inhibition. It is possible, therefore, that the observed effects could simply arise following activation of