An effect of cannabis treatment *in vivo* on noradrenaline-stimulated lipolysis in rat adipocytes

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The active principles of cannabis and its metabolites are known to concentrate in body fat during chronic administration (Kreuz & Axelrod, 1973; Forney & Kiplinger, 1971), and in the plasma they are strongly bound to lipoprotein (Wahlqvist, Nilsson & others, 1970; Widman, Nilsson & others, 1973). Such lipophilic properties of the cannabinoids point to lipid-containing or lipid-involved structures for possible sites of action. Further, a striking depletion of fat stores in rats receiving oral Δ^1-tetrahydrocannabinol (Δ^1-THC) over a period of weeks was observed by Braude (1972), and Paton (unpublished work) found a great reduction in visible body fat in mice receiving oral cannabis for up to five weeks.

The present report, part of a preliminary study of the effects of cannabis *in vivo* on various aspects of fat metabolism is concerned with the lipolytic activity of rat adipocytes.

Male, albino rats of the Wistar strain, 150–190 g in the fed state, were given by gavage cannabis (tincture of cannabis B.P.C. 1949: W. Ransom, Hitchin, Herts.) as a dose of 150 mg crude marihuana extract kg^{-1}, yielding a Δ^1-THC dose of 5·4 mg kg^{-1}. This route was used by Braude (1972) and by Paton (see above) and also avoids the local irritant effect of cannabis parenteral injections. The resin was given dispersed in approximately 2 ml milk while the rats were under light ether anaesthesia. Doses were given daily for two days. For studies on lipolysis it is necessary to standardize the nutritional state of the animals: the second dose therefore contained glucose (20% w/v), after which the rats were starved for 24 h before death. Control animals received pure milk (and glucose, when applicable). There were no significant differences either in food and water intake between the control and cannabis-treated animals or in the combined weights of the two epididymal fat bodies at death (665·3 mg ± 50·4 [s.e.m.] for the control rats, and 633·4 mg ± 46·6 [s.e.m.] for the cannabis-treated). During this period the rats were housed at 22°. Experiments were limited to the crude marihuana extract since this is the form normally used by man and the constituent cannabinoids can interact (Jones & Pertwee, 1975). The dose of cannabis used is within the range of human use (Fairbairn, Hindmarch & others, 1974) particularly in view of its lower bioavailability by the oral route (Garrett & Hunt, 1974).

The results in Table 1 show that in albumin-free incubations, fat-cells from cannabis-treated rats have a significantly higher lipolytic rate (+36%) than cells from the controls in the presence of 1 μM noradrenaline, a concentration which gave an intermediate stimulation of lipolysis. The effect was not increased at other concentrations of noradrenaline. In the presence of albumin overall rates of lipolysis in both groups are much higher with 1 μM noradrenaline (Table 1) but are not significantly different from each other; no significant differences were found at other noradrenaline concentrations with albumin.

The conditions for availability of albumin *in vivo* as an acceptor of free fatty acids (FFA) produced by lipolysis probably come between those *in vitro* for fat cells incubated either with or without albumin. The generally lower rates of lipolysis seen in the absence of albumin *in vitro*, however, may be more directly relevant to the situation *in vivo* since rates of this magnitude occur with *intact* adipose tissue, whether incubated or perfused with an albumin-containing medium (Scow, Stricker & others, 1965; Rodbell, 1965). The significant difference in lipolytic response now reported in this *in vitro* situation shows that an effect of cannabis treatment *in vivo* was retained by the cells during their isolation and subsequent incubation.

Additional experimental evidence has eliminated several possible explanations for the effect. Thus; (i) no direct effects of cannabinoids retained in the adipose

Table 1. *Effects of noradrenaline and albumin on lipolytic activities of fat cells from control and cannabis-treated rats.* Fat cells were isolated (Rodbell, 1964) from the epididymal fat bodies of at least two treated rats and were incubated at 37° in Krebs-Ringer bicarbonate buffer (20 mg cells, dry wt ml^{-1}), equilibrated with $O_2 + CO_2$ (95 : 5) and containing 6 mM glucose. Noradrenaline (Koch-Light Labs., Colnbrook, Bucks) was added as the bitartrate and albumin (Fraction V from bovine plasma: Armour, Eastbourne) was previously dialysed against the buffer. Glycerol was assayed according to Garland & Randle (1962). Incubations were for 30 min to ensure linearity of glycerol release in the absence of albumin (see also Knight & Iliffe, 1973). Mean values ± s.e.m. are quoted with the number of observations in parenthesis.

Noradrenaline	Albumin (4% w/v)	Lipolysis (μM glycerol/100 mg cells h^{-1}) Control	Cannabis-treated
—	—	0·54 ± 0·04 (15)	0·74 ± 0·10 (13)
1 μM	—	1·01 ± 0·06 (15)	1·37 ± 0·11 (13)*
1 μM	+	8·71 ± 1·04 (5)	9·08 ± 0·73 (4)

* Significantly different from control, $P<0.01$ (Student's t-test).

* Correspondence.

tissue seems likely since we have found that if the drug is added *in vitro* to incubations containing adipose tissue from normal rats, it had no effect either on basal or on noradrenaline-stimulated lipolysis. This agrees with the lack of effect of a number of cannabinoids on basal lipolysis *in vitro*, reported by Malor, Jackson & Chesher (1978), when the drugs were added to incubations of mouse adipocytes. (ii) No effect attributable to a fall in body temperature produced by cannabis was possible since if the rats were housed at 28° instead of 22° a similar increase in response to noradrenaline in the absence of albumin was obtained. Further, the effect could not be due to a change in the nutritional state of the animal, since the procedures used were successful in controlling this (see above). (iii) A further explanation is that in the cannabis-treated rats there was an increase in the number of fat cells per unit weight of cells. If so, similar effects, on a percentage basis, might have been expected whether albumin was present or absent. Moreover, from other evidence (Zinder & Shapiro, 1971; Hubbard & Matthew 1971; Gorman, Tepperman & Tepperman, 1972), a small decrease in cell diameter, necessary to increase the observed lipolytic rate by 36% when expressed per unit of triglyceride concentration or per unit of fat-cell weight, can be estimated to cause a decrease in the fresh weight of the whole fat pad at least several fold larger than the 5% decrease (not significant) observed in this study. The possibility that cannabis caused a compensatory increase in the fat-cell number per fat pad is inconsistent with the known effects of the drug on cell division and DNA metabolism (Nahas, Desoize & others, 1976).

The possibility cannot yet be excluded that the stimulated fat cells from the cannabis-treated rats are able to reduce the anticipated rise in intracellular FFA concentrations and so to decrease the inhibitory effect of FFA on the lipolytic process. With glucose present in the medium further protection from high intracellular FFA concentrations should be available (Angel, Desai & Halperin, 1971). Such protection would not be required in the presence of albumin *in vitro*. Recent reports (Maier & Maître, 1975; Malor, Chesher & Jackson, 1976; Malor & others, 1978) have shown that acute effects of Δ^1-THC cause an elevation of plasma FFA concentrations in rats and mice and that this is probably centrally mediated. The present work indicates that after a longer period of administration of the crude marihuana extract, the fat cells from such rats are themselves affected by the drug or its metabolites. The effect is a small one under the conditions of these experiments, but exerted over a period of weeks could contribute to the losses of fat recorded.

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REFERENCES

ANGEL, A., DESAI, K. S. & HALPERIN, M. L. (1971). *J. Lipid Res.*, **12**, 203–213.

BRAUDE, M. C. (1972). In: *Cannabis and its derivatives*, p. 88–100. Editors: Paton, W. D. M. & Crown J., London: Oxford University Press.

FAIRBAIRN, J. W., HINDMARCH, I., SIMIC, S. & TYLDEN, E. (1974). *Nature, Lond.*, **249**, 276–278.

FORNEY, R. B. & KIPLINGER, G. F. (1971). *Ann. N.Y. Acad. Sci.*, **191**, 74–82.

GARLAND, P. B. & RANDLE, P. J. (1962). *Nature, Lond.*, **196**, 987–988.

GARRETT, E. R. & HUNT, C. A. (1974). *J. Pharm. Sci.*, **63**, 1056–1064.

GORMAN, R. R., TEPPERMAN, H. M. & TEPPERMAN, J. (1972). *J. Lipid. Res.*, **13**, 276–280.

HUBBARD, R. W. & MATTHEW, W. T. (1971). *Ibid.*, **12**, 286–293.

JONES, G. & PERTWEE, R. G. (1975). *Br. J. Pharmac.*, **45**, 375–377.

KNIGHT, B. L. & ILIFFE, J. (1973). *Biochem. J.*, **132**, 77–82.

KREUZ, D. S. & AXELROD, J. (1973). *Science*, **179**, 391–393,

MAIER, R. & MAÎTRE, L. (1975). *Biochem. Pharmac.*, **24**, 1695–1699.

MALOR, R., CHESHER, G. B. & JACKSON, D. M. (1976). *J. Pharm. Pharmac.*, **28**, 652–654.

MALOR, R., JACKSON, D. M. & CHESHER, G. B. (1978). *Biochem. Pharmac.*, **27**, 407–413.

NAHAS, G. G., DESOIZE, B., HSU, J. & MORISHIMA, A. (1976). In: *Marihuana. Chemistry, Biochemistry and Cellular Effects*, p. 299–312. Editor: Nahas, G. G., New York, Heidelberg, Berlin: Springer-Verlag.

RODBELL, M. (1964). *J. biol. Chem.*, **239**, 375–380.

RODBELL, M. (1965). *Ann. N.Y. Acad. Sci.*, **131**, 302–314.

SCOW, R. O., STRICKER, F. A., PICK, T. Y. & CLARY, T. R. (1965). *Ibid.*, **131**, 288–301.

WAHLQVIST, M., NILSSON, I. M., SANDBERG, F., AGURELL, S. & GRANSTRAND, B. (1970). *Biochem. Pharmac.*, **19**, 2579–2584.

WIDMAN, M., NILSSON, I. M., NILSSON, J. L. G., AGURELL, S. BORG, H. & GRANSTRAND, B. (1973). *J. Pharm. Pharmac.*, **25**, 453–457.

ZINDER, O. & SHAPIRO, B. (1971). *J. Lipid Res.*, **12**, 91–95.