The effect of Δ^9-tetrahydrocannabinol on plasma concentrations of non-esterified fatty acids in the mouse

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Askew & Ho (1974) reported that Δ^8-tetrahydrocannabinol (Δ^8-THC) may produce changes in concentrations of cyclic adenosine 3'5'-monophosphate (cAMP) in rat brain. In this communication we present some indirect evidence to support this finding, using lipolysis as a model system. We have noted the effects of Δ^9-tetrahydrocannabinol (THC) *in vivo* on the concentrations of plasma non-esterified fatty acids (NEFA), and have compared these effects to those produced by adrenaline.

Female QS strain mice (25–30g) were used and were kept in groups of about 25 and maintained on a 12 h light-dark (non-reversed) cycle. Food and water were freely available up to the time of experimentation. During the experiments the animals were handled as gently as possible and laboratory noise reduced to a minimum. THC (molecular weight = 314·45), dissolved and stored at −40° in propylene glycol for a maximum of one week, was prepared as a suspension (before each experiment) in propylene glycol and Lissapol-Dispersol (ICI; Whittle, 1964). The final propylene glycol concentration was 5%. (±)-Adrenaline bitartrate (Sigma) was dissolved in 10% ascorbic acid and (±)-propranolol hydrochloride (ICI) was dissolved in 0·9% saline. The animals were dosed with THC either by gavage or by intraperitoneal injection, all of the other drugs being given intraperitoneally. Control animals were dosed by the stated route with the appropriate vehicle. A dose-volume of 10 ml kg^{-1} was used. The mice were decapitated and blood was collected in citrated tubes. The plasma NEFA were determined by the titrimetric method of Dole (1956).

Adrenaline, calculated as the base, 5·459 μmol kg^{-1} (i.p.), produced an immediate rise in plasma NEFA which reached a maximum 5 min after injection and virtually returned to the pre-dose concentration after 15 min (Fig. 1). THC, 3·180 μmol kg^{-1} (i.p.), produced an increase in the plasma NEFA which was lower than that elicited by adrenaline and which peaked 30 min after injection, returning to the basal concentration by 60 min. When given by gavage THC (3·180 μmol kg^{-1}) produced a peak rise in NEFA between 15 and 30 min and this increase was maintained for at least 60 min.

Dose-response curves for THC (dose range 0·080 to 3·180 μmol kg^{-1}) and adrenaline (0·136 to 5·459 μmol kg^{-1}) were determined after intraperitoneal administration and NEFA concentrations determined 5 min after adrenaline and 30 min after THC (Table 1). The THC vehicle itself produced a significantly higher plasma NEFA than did the adrenaline vehicle. Even the lowest dose of THC (0·080 μmol kg^{-1}) resulted in a significant increase (Student's t-test, $P < 0.05$) in lipolysis, though the maximum response produced by THC was less than that produced by adrenaline. When the dose-response curves (corrected for the vehicle response) were compared it could be seen that at least at the lower doses, THC was approximately equipotent with adrenaline.

To study the effect of β-adrenoceptor blockade on these responses, propranolol (calculated as base), 19·290 μmol kg^{-1} (i.p.), was given 25 min before adrenaline (0·546 μmol kg^{-1}) or simultaneously with THC (0·318 μmol kg^{-1}). Both these doses produced a significant elevation of NEFA, and the animals were killed 5 and 30 min after adrenaline or THC respectively. These times were at the peak of the drug action (Fig. 1 and Table 2). While propranolol completely abolished the response to adrenaline it was without effect on the response to THC. A further experiment was carried out to control against the possibility that the absorption of propranolol might have been slower than that of the THC. Propranolol was injected 25 min before THC and plasma NEFA determined 30 min after the latter injection (Table 2, Expt. B). As before, propranolol failed to block the THC-induced increase in NEFA.

The mobilization of fatty acids from adipose tissue by the action of adipose tissue lipases has been shown to be sensitive to a wide variety of biologically active compounds including sympathomimetic amines and a number of hypophyseal hormones (Schotz & Page, 1960; Stock & Westermann, 1965; Fain, Galton &

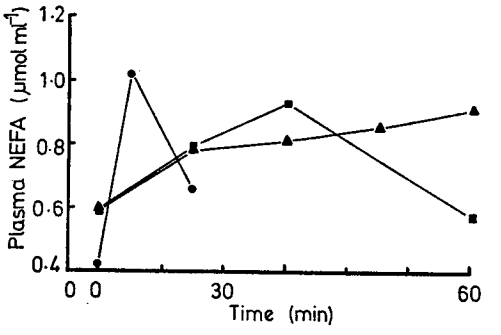

FIG. 1. The time-response curves of 5·459 μmol adrenaline kg^{-1}, intraperitoneally ● — ● and 3·180 μmol THC kg^{-1}, intraperitoneally ■ — ■ or by gavage ▲ — ▲ on the concentrations of plasma non-esterified fatty acids in the mouse. Each point represents the mean value of at least 6 experiments. In all cases the s.e.m. were less than 7% of the mean value, and the error bars have been omitted for clarity.

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Table 1. *Dose-response curves for THC and adrenaline on concentrations of mouse plasma non-esterified fatty acids.*

Treatment	Drug (μmol kg^{-1}) Adrenaline	THC	n	Plasma NEFA (μmol ml^{-1}) mean ± s.e.m.
Vehicle	—	—	16	0·423 ± 0·018
Adrenaline	0·136	—	18	0·493 ± 0·052
Adrenaline	0·273	—	10	0·584 ± 0·068
Adrenaline	0·409	—	12	0·679 ± 0·029
Adrenaline	0·546	—	12	0·822 ± 0·073
Adrenaline	1·365	—	5	0·983 ± 0·091
Adrenaline	2·729	—	11	0·981 ± 0·075
Adrenaline	4·094	—	6	1·253 ± 0·055
Adrenaline	5·459	—	6	1·186 ± 0·276
Vehicle	—	—	75	0·593 ± 0·020
THC	—	0·080	11	0·714 ± 0·049
THC	—	0·159	12	0·649 ± 0·045
THC	—	0·239	15	0·803 ± 0·036
THC	—	0·318	30	0·766 ± 0·033
THC	—	1·590	11	0·991 ± 0·068
THC	—	3·180	18	0·949 ± 0·053

The plasma NEFA were determined 5 min after the adrenaline injection and 30 min after the THC injection.

Table 2. *The effect of propranolol pretreatment on the lipolytic action of THC and adrenaline.*

Treatment	Drug (μmol kg^{-1}) Propranolol	Adrenaline	THC	n	Plasma NEFA (μmol ml^{-1}) mean s.e.m.
Expt. A					
Vehicle	+	—	—	5	0·275 ± 0·027
Adrenaline	+	0·546	—	6	0·327 ± 0·026
Adrenaline	—	0·546	—	6	0·856 ± 0·110
Expt. A					
Vehicle	+	—	—	5	0·658 ± 0·055
THC	+	—	0·318	6	0·819 ± 0·106
THC	—	—	0·318	6	0·863 ± 0·066
Expt. B					
Vehicle	+	—	—	6	0·508 ± 0·031
THC	+	—	0·318	11	0·709 ± 0·036
THC	—	—	0·318	12	0·793 ± 0·045

Dose of propranolol base used: 19·290 μmol kg^{-1}. In Expt. A the animals were killed 5 min after the adrenaline and 30 min after the administration of the THC. Propranolol was given 25 min before the adrenaline and simultaneously with the THC. In expt. B the animals were killed 30 min after the THC and propranolol was administered 25 min before the injection of THC.

Kovacev, 1966). The lipolytic action of these agents has been demonstrated both *in vivo* and *in vitro*, and they all probably act by stimulating the formation of cAMP by adenyl cyclase (Rodbell, Birnbaumer & Pohl, 1969).

In the present study we have shown that THC exerts a lipolytic effect of comparable potency to that of adrenaline. Both drugs showed a dose dependent response, with the dose-response curves being approximately parallel over the lower dose ranges, and the maximum response produced by THC being about 80% of that produced by adrenaline. As expected, the duration of action of adrenaline was very short due to its rapid degradation. The longer duration of action of THC might be attributed to a number of factors including its slower rate of absorption, metabolism, its strong protein binding (Wahlqvist, Nilsson & others, 1970; Widman, Nilsson & others, 1973) and its uptake and storage in lipid rich areas (Kreuz & Axelrod, 1973). When given by gavage THC produced an effect of much longer duration than the same dose given intraperitoneally. The reason for this difference is not known but a number of factors are possibly involved. Following intraperitoneal injection the THC directly bathes the mesenteric and perirenal fat stores and much of the administered dose would be taken up immediately into those stores, thus shortening the duration of action of the drug. However, with the orally administered THC the entry of the drug into the system would be more continuous and extend over a longer time.

The vehicle used to suspend the THC showed a positive lipolytic activity. Indeed, it has been reported that most of the surface-active agents used to suspend THC often exert an intrinsic activity (Sofia, Kubena & Barry, 1971)†. The adrenaline vehicle (10% ascorbic acid) showed no lipolytic activity.

Of considerable importance was our finding that propranolol was completely ineffective in blocking the fat mobilizing action of THC in doses which completely antagonized the effect of adrenaline. This would suggest that THC and adrenaline are acting at different sites to produce their lipolytic effects. At least two mechanisms of action of THC may be hypothesized. Firstly, THC could act directly on adipose tissue, possibly by stimulating the adenyl cyclase of that tissue. It is well documented that THC can inhibit the synthesis of prostaglandins (PG's) (Burstein & Raz, 1972; Burstein, Levin & Varanelli, 1973). Moreover, Askew & Ho (1974) have reported that Δ^8-THC can elicit changes in cAMP, adenyl cyclase and phosphodiesterase. It has been further shown that THC is able to antagonize PGE_1-induced elevation of cAMP in cultured WI-38 fibroblasts (Kelly & Butcher, 1973). It has been postulated that PG's may play a role in the control of lipolysis by exerting feedback inhibition of the synthesis of cAMP in stimulated adipose tissue (for review see Silver & Smith, 1975). It could follow therefore, that the presence of a drug such as THC, which can inhibit the formation of PG's from released arachidonic acid, could interfere with the feedback inhibition loop on cAMP synthesis and thus lead to an elevation in plasma NEFA. In support of this hypothesis we have evidence that THC may have a direct action on the proposed PGE_1 receptor site, and that this action follows first order kinetics (Jackson, Malor & others, 1976).

The second hypothesis is that THC could act indirectly via the release of hypophyseal and/or adrenal cortical hormones. Maier & Maitre (1975) reported recently (while the present work was being completed) that THC depletes the rat adrenal gland of both ascorbic acid and cholesterol esters in a fashion comparable to that produced by ACTH. Furthermore, these authors noted a concomitant rise in both plasma corticosterone and NEFA. These effects were abolished in adrenalectomized animals. However, two points should be noted. Firstly, the authors do not state whether the animals were kept at a thermally neutral temperature. It is well documented that at the dose of THC used (30 mg kg^{-1}), the animals would exhibit marked hypothermia (Holtzmann, Lovell & others, 1969; Abel

1972; Anderson, Jackson & others, 1975). This could well have resulted in a 'stress reaction' which might be expected to elicit the responses which have been described. At the doses used in the present study there was no significant hypothermic response (Anderson & others, 1975). The second point of criticism of the paper of Maier & Maitre (1975) lies again in the very high dose of THC used (30 mg kg^{-1})—a dose which was between 60 and 1200 times as high as that required in the present work to produce a significant rise in plasma NEFA. Nevertheless, the data reported by these authors are compatible with our findings since ACTH would produce a rise in plasma NEFA which would resist blockade by β-adrenoceptor antagonists. Moreover, these authors found a similar time course of the effect of THC on plasma NEFA to that reported here.

The two hypotheses presented are not necessarily mutually exclusive, since both a THC-induced release of ACTH as well as a direct action of THC at peripheral sites may occur.

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† Sofia & others (1971, loc. cit.) found that of four different vehicles used to suspend THC, the vehicle which possessed the least intrinsic activity on the system studied (electroshock seizure) consisted of 10% propylene glycol, 1% Tween 80 and 89% isotonic saline. Accordingly this vehicle was tested using the present system. The vehicle alone resulted in plasma NEFA concentration of 0·583 ± 0·025 μmol ml^{-1} (n = 33) and this was not significantly different from that observed with the propylene glycol—Lissapol vehicle (0·593 ± 0·020 μmol ml^{-1}, n = 81). Moreover, no difference was observed in the lipolytic response to THC when suspended in either vehicle. Thus THC, 0·318 μmol kg^{-1}, suspended in propylene glycol—Tween—saline produced a plasma NEFA of 0·685 ± 0·022 μmol ml^{-1} (n = 26), while the same dose of THC, suspended in the propylene glycol - Lissapol vehicle gave a value of 0·748 ± 0·047 μmol ml^{-1} (n = 18).

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