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POSSIBLE CENTRAL DOPAMINERGIC MODULATION OF THE RISE IN PLASMA CONCENTRATION OF NON-ESTERIFIED FATTY ACIDS PRODUCED IN THE MOUSE BY (−)*TRANS*-Δ^9-TETRAHYDROCANNABINOL

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Abstract—Δ^9-Tetrahydrocannabinol (THC), 11-OH THC, 8α,11-diOH THC, cannabinol (CBN) and cannabidiol (CBD) were examined for direct lipolytic activity on mouse adipocytes *in vitro*. None of the cannabinoids showed any marked stimulation of lipolysis, nor did they modify the response of the adipocytes to either isoprenaline or ACTH. *In vivo*, THC (but not CBN or CBD) produced in mice a dose-dependent rise in plasma non-esterified fatty acids (NEFA). This response was prevented by prior bilateral adrenalectomy or by pretreatment with α-methyl-*p*-tyrosine. Pretreatment with FLA-63 or phentolamine did not alter the lipolytic response to THC. However, the THC-induced rise in plasma NEFA was blocked by prior administration of the dopamine receptor antagonists perphenazine or pimozide. It is suggested that the elevation of plasma NEFA produced in mice by THC is centrally mediated and requires the presence of functional dopaminergic receptors.

A number of reports have suggested that (−)*trans* Δ^9-tetrahydrocannabinol (THC),† the major psychoactive component of *Cannabis sativa*, may affect the hypothalamic-pituitary-adrenal axis. Kubena *et al.* [1] found that THC (1–4 mg/kg) produced a dose-dependent increase in plasma corticosterone levels in rats, and showed that prior hypophysectomy abolished this effect. Others workers confirmed these results [2, 3] and also reported that THC (and Δ^8-THC) depleted adrenal cholesterol esters and ascorbic acid and increased the plasma non-esterified fatty acid (NEFA) levels in rats [2]. All of these effects were abolished by prior hypophysectomy, but not by adrenalectomy. This latter finding led Maier and Maître [2] to hypothesize that THC stimulated the release of ACTH by an action on either the pituitary or the hypothalamus.

Recently we reported [4] that THC produces a marked increase in plasma NEFA levels when given to mice in low doses (0.025 to 1.0 mg/kg), and that in this respect THC has a potency comparable to adrenaline. However, the lipolytic effect of THC *in vivo* (unlike that of adrenaline) was not abolished by propranolol pretreatment [4]. Because of these reports which suggested an interaction between THC and the hypothalamic–pituitary–axis, we decided to investigate this interaction further by examining the effect of various pharmacological pretreatments on the ability of THC (and other cannabinoids) to elevate plasma NEFA levels in mice. In addition, because we had previously hypothesized [4] that THC could conceivably elicit this response by a direct effect on adipose tissue, we also investigated its lipolytic action on isolated mouse adipocytes.

MATERIALS AND METHODS

Animals. QS strain female mice (25–30 g), kept in groups of about 25, were used in all experiments. They were kept at 21 ± 2° on a 12 hr light, 12 hr dark cycle (non-reversed) for at least 3 days prior to use, and allowed food and water freely up to the time of experimentation. During experimentation, in order to control for stressful stimuli, the animals were handled as gently as possible and laboratory noise was reduced to a minimum.

Methods in vitro. Isolated adipocytes were prepared essentially by the method of Rodbell (as described by Fain [5]). Basically, pieces of mesenteric and perirenal fat were incubated in the incubation medium (4% BSA in a modified Krebs–Henseleit solution without glucose) which contained 0.5 mg collagenase/g of tissue. The incubation proceeded for 45 min at 36 ± 0.5° with constant shaking. At the end of the incubation, the material was filtered under gentle positive pressure through several layers of surgical nylon gauze. The fat cells were washed four times by flotation in 10 ml of the incubation medium with mild centrifugation (200 *g* for 15 sec). The packed cells were finally resuspended at an approximate concentration of 200 mg of tissue equivalents/ml in the incubation medium which had been saturated with carbogen. Aliquots of 1 ml were incubated at 37.5 ± 0.5° for 3 hr with constant shaking and air as the gas phase. At the end of the incubation time, tubes were mixed on a vortex mixer and aliquots of 0.2 ml were taken for NEFA assay by the method of Dole [6]. All incubations were carried out in duplicate or triplicate. Cannabinoids were added in 1 μl ethanol, isoprenaline in

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† Abbreviations used in the text are as follows: THC, (−)*trans* Δ^9-tetrahydrocannabinol; ACTH, adrenocorticotrophic hormone; α-MT, α-methyl-*p*-tyrosine; BSA, bovine serum albumin; cAMP, cyclic adenosine 3′,5′-monophosphate; CBD, cannabidiol; CBN, cannabinol; CRF, corticotrophin releasing factor; DA, dopamine; and NEFA, non-esterified fatty acids.

10 μl of 15.5 mM ascorbic acid, and ACTH in modified Krebs–Henseleit medium.

In the interaction studies, the cannabinoids (or vehicle) were always added immediately before the addition of the other drugs. The addition of 1 μl ethanol did not affect the lipolysis produced by isoprenaline or ACTH. In all experiments duplicate tubes containing 10^{-6} M isoprenaline were incubated, together with duplicates containing no drugs (but with ethanol and/or ascorbic acid vehicle) to give the 100 per cent and basal response points, respectively, for the individual adipocyte preparation. In the experimental tubes, the data were expressed as a percentage of the maximal stimulation of the preparation elicited by 10^{-6} M isoprenaline or 10^{-6} M ACTH because the sensitivity of each individual adipocyte preparation varied in its response to known lipolytic agents (e.g. see Ref. 7).

Methods in vivo. The animals were premedicated appropriately as described below in Results. Some mice were bilaterally adrenalectomized under ether anaesthesia 24 hr prior to use. During this time they were given a saline supplement. The mice were decapitated, and the blood from each mouse was collected in a tube containing approximately 20 mg sodium citrate, and plasma NEFA was determined [6].

Drugs. THC (N.I.M.H.) was obtained as an ethanolic solution, all of the other cannabinoids being supplied in powder form (N.I.M.H.). For experiments *in vivo*, the THC solution was dried at 20° under nitrogen and dissolved and stored at −40° in propylene glycol for a maximum of 7 days. Stock solutions of CBD and CBN were similarly prepared and stored in propylene glycol. For administration to the animals, THC, CBD and CBN were prepared as suspensions before each experiment to provide in all cases 10% v/v propylene glycol, 1% v/v Tween 80 and 89% v/v normal saline. For studies *in vitro*, THC, 11-OH THC (N.I.M.H.), 8α,11-diOH THC (N.I.M.H.), CBD and CBN were dissolved directly in ethanol. Collagenase (Type II from *Clostridium histolyticum*, Sigma) was dissolved in 4% BSA–Krebs–Henseleit solution immediately before use. The BSA (Sigma, Fraction V) was purified before use [5]. Perphenazine dihydrochloride (prepared from perphenazine base, Schering Corp.) and α-methyl-*p*-tyrosine methyl ester hydrochloride (α-MT, Sigma) were dissolved in normal saline. *dl*-Isoprenaline hydrochloride (Sigma) was dissolved in 15.5 mM ascorbic acid. Phenoxybenzamine hydrochloride (Smith, Kline & French), FLA-63 [bis-(4-methyl-1-homopiperazinylthiocarbonyl) disulfide] (A. B. Hässle) and pimozide (Ethnor) were dissolved in a few drops of glacial acetic acid and adjusted to volume with saline. *dl*-Adrenaline bitartrate (Sigma) was dissolved in 10% ascorbic acid. For studies *in vivo*, adrenocorticotrophic hormone (ACTH) was used in the form of a gel (ACTHAR gel, Armour) appropriately diluted with saline, and for studies *in vitro* ACTH pure substance (CIBA-Geigy) was dissolved in modified Krebs–Henseleit solution. Phentolamine mesylate was used in ampoule form (Regitine, Ciba) and diluted with saline. Control animals for particular experiments were dosed with the appropriate vehicle solution. All drugs were administered i.p. in a dose volume of 10 ml/kg, and the drug doses given in the text refer to the forms above except for isoprenaline hydrochloride and adrenaline bitartrate, which have been expressed as the base.

RESULTS

Experiments in vitro. None of the five cannabinoids tested exhibited any marked direct lipolytic effect when incubated with isolated mouse adipocytes (Table 1). In all cases, the change in NEFA release was not more than 6 per cent higher than the normal basal NEFA increase which occurred over the 3-hr incubation period, and this basal NEFA increase was considered to be at the threshold sensitivity of the experimental procedure. The cannabinoids did not produce lipolysis in the presence of ascorbic acid (1.53×10^{-4} M final concentration, data not shown) despite the fact that ascorbic acid has been reported to shift the dose–response curve of isoprenaline-

Table 1. Effect of five different cannabinoids tested for intrinsic lipolytic action on isolated mouse adipocytes*

Drug conc n (M)	Cannabinoid (Response as per cent of response to isoprenaline, 10^{-6} M)				
	THC	11-OH THC	8α,11-diOH THC	CBN	CBD
10^{-10}	2.97 (7)	−0.12 (2)	1.88 (2)		
10^{-9}	−0.51 (2)				
10^{-8}	1.83 (3)	−1.38 (2)	−0.56 (2)		
10^{-7}	5.99 (2)		3.17 (1)		
10^{-6}	1.31 (3)	−0.83 (1)			
10^{-5}	3.20 (2)				
10^{-4}	1.44 (3)			1.6 (1)	3.3 (1)

* The results are expressed as a percentage of the response of the preparations to a maximal dose (10^{-6} M) of isoprenaline (expressed as the base). The numbers in brackets represent the number of experiments. In each experiment, duplicate or triplicate tubes were incubated and there was a high degree of consistency between experiments.

The range of absolute NEFA release lay between 1400 and 2800 nmoles NEFA/ml/3 hr (with basal release subtracted) for isoprenaline (10^{-6} M). The average NEFA release for this concentration of isoprenaline for the 3 hr incubation in twelve typical experiments was 1921 ± 169 nmoles/ml.

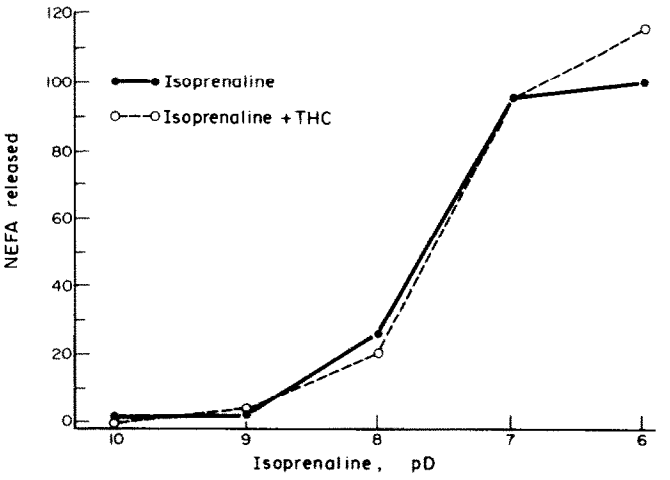

Fig. 1. Effect of incubation with THC (10^{-4} M) on the response of isolated mouse adipocytes to the lipolytic effect of isoprenaline. Each point represents the mean of two experiments, each of which was performed in triplicate. The isoprenaline concentration is expressed as *pD* (i.e. log M concentration). The NEFA released is expressed as a percentage of that released by isoprenaline 10^{-6} M.

induced lipolysis to the left by about 0.57 log units [7].

When either isoprenaline or ACTH was used as an agonist (concentration range for both was 10^{-10} to 10^{-6} M), THC, even at 10^{-4} M, did not affect the shape or position of the dose–response curves (Figs. 1 and 2). The ethanol vehicle for the THC also had no effect on isoprenaline or ACTH-induced lipolysis. CBN, CBD, 11-OH THC and 8α,11-diOH THC were tested at concentrations between 10^{-4} and 10^{-8} M in combination with maximal concentrations (10^{-6} M) of both ACTH and isoprenaline, and were found to be ineffective in altering the lipolytic response of the adipocytes to these agonists (data not shown).

Experiments in vivo. THC (0.08 to 6.36 μmoles/kg, i.e. 0.025 to 2.0 mg/kg), ACTH (0.05 to 8.0 IU/kg) and adrenaline (0.136 to 5.459 μmoles/kg, i.e. 0.1 to 3.0 mg/kg) administered i.p. all produced dose-dependent increases in plasma NEFA as measured at the time of peak drug action (30 min after THC injection, 15 min after ACTH and 5 min after adrenaline [4, 8] Fig. 3). The maximum responses to ACTH (1068 ± 52 nmoles/ml) and to THC (991 ± 68 nmoles/ml) were not significantly different ($P > 0.05$), but both were lower ($P < 0.05$) than the response elicited by adrenaline (1250 ± 52 nmoles/ml). The adrenaline vehicle produced a significant ($P < 0.001$) lowering in basal NEFA levels compared to the vehicles used for THC and ACTH (Fig. 3). Neither CBD (3.22, 9.66 and 32.2 μmoles/kg) nor CBN (3.18, 9.54 and 31.8 μmoles/kg) administered i.p. produced any significant changes in basal NEFA levels (Table 2, $P > 0.05$), although there was a dose-dependent though non-significant increase obtained with CBD. THC (1.59 to 6.36 μmoles/kg) caused an elevation ($P < 0.01$) which was similar to

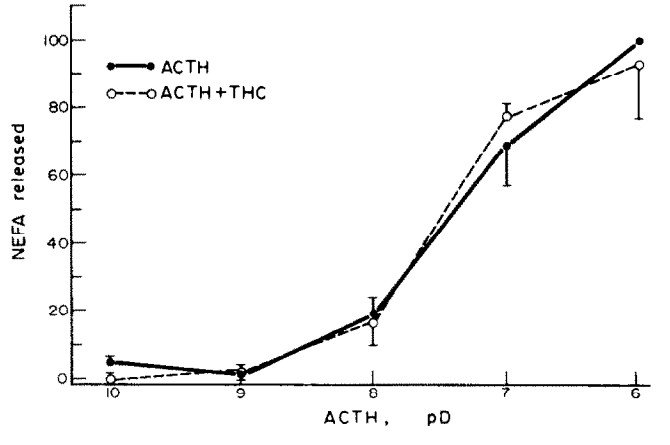

Fig. 2. Effect of incubation with THC (10^{-4} M) on the response of isolated mouse adipocytes to the lipolytic effect of ACTH. Each point represents the mean of four experiments, and the vertical bars represent the S. E. M. Each experiment was performed in duplicate. The ACTH concentration is expressed as *pD* (i.e. log M concentration). The NEFA released is expressed as a percentage of that released by ACTH 10^{-6} M.

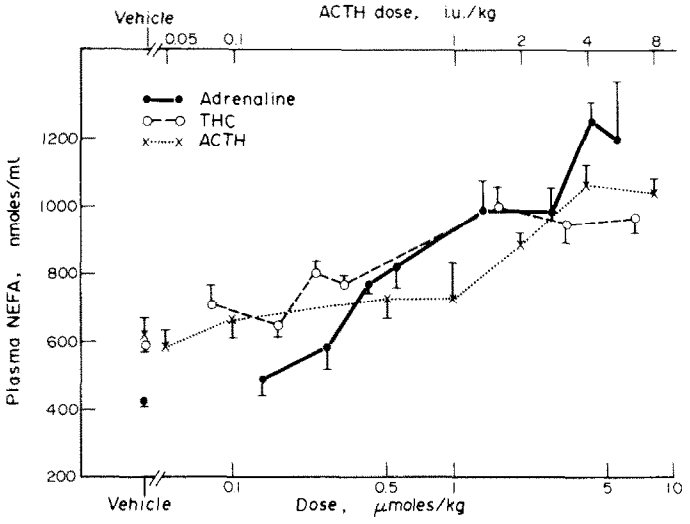

Fig. 3. Effect of i.p. administration of various doses of adrenaline, THC and ACTH on the plasma levels of NEFA in mice. Mice were killed 5, 30 and 15 min after adrenaline, THC or ACTH respectively. Each point represents the mean of between eight and thirty experiments, and the vertical bars represent the S. E. M.

that seen in Fig. 3 and which was already maximal at a dose of 1.59 μmoles/kg, in agreement with the data in Fig. 3.

Since we had previously reported [4] that the lipolytic effect of THC was not blocked by the β-adrenergic receptor antagonist propranolol, it appeared that the adrenal medullary catecholamines were not of critical importance in this response. Ablation of the adrenal glands, however, prevented the elevation of plasma NEFA by either 0.318 or 3.18 μmoles/kg of THC (Table 3). Since THC (0.318 μmole/kg) was active in the adrenalectomy controls, these data suggest that an intact adrenal gland was required.

FLA-63 altered neither the basal NEFA levels (Table 4) nor the stimulatory effect of THC (3.18 μmoles/kg) (Table 4). In contrast to FLA-63, pretreatment with α-MT induced a non-significant depression (Table 4) of basal NEFA levels. THC (3.18 μmoles/kg) elevated the plasma NEFA (Table 4) and this rise was markedly depressed by prior administration of α-MT (Table 4). Pretreatment with α-MT and THC only raised the NEFA levels to those which were seen in the vehicle-treated animals. Using a paired *t*-test, the mean difference ± S. E. M. between (1) the α-MT vehicle plus THC vehicle group and the α-MT vehicle plus THC group and (2) the α-MT plus THC vehicle group and the α-MT plus THC group was, respectively, 0.4761 ± 0.0773 (10 animals) and 0.1275 ± 0.0456 (9 animals), $t = 3.8842$, $df = 17$, $P = < 0.01$, indicating that there had been a significant inhibition of the THC response by α-MT.

Blockade of α-adrenergic receptors with phentolamine did not alter the response to THC (Table 5) although phentolamine by itself showed intrinsic activity (Table 5). Perphenazine and pimozide blocked the lipolytic effect of THC. Perphenazine itself had no effect on basal NEFA levels (Table 5), and in animals pretreated with perphenazine, THC did not produce any change in this level (Table 5). Pimozide exhibited a weak, though non-significant intrinsic lipolytic

Table 2. Effect of i.p. administration of various doses of THC, CBD and CBN on the plasma levels of NEFA in mice*

Treatment dose (μmoles/kg)	Plasma NEFA levels (nmoles/ml)	No. of animals
Vehicle	608 ± 62	13
CBD (3.22)	616 ± 55	8
CBD (9.66)	622 ± 75	10
CBD (32.2)	687 ± 73	9
CBN (3.18)	673 ± 110	7
CBN (9.54)	682 ± 68	9
CBN (31.80)	675 ± 51	11
THC (1.59)	994 ± 65	7
THC (3.18)	927 ± 98	7
THC (6.36)	925 ± 81	9

* Mice were killed 30 min after cannabinoid administration.

Table 3. Effect of bilateral adrenalectomy upon the response to THC (0.318 and 3.18 μmoles/kg, i.p.)-induced lipolysis in mice*

Treatment	Plasma NEFA levels (nmoles/ml)	No. of animals
Adrenalectomy plus THC vehicle	549 ± 35†,‡	10
Adrenalectomy plus THC (0.318 μmole/kg)	608 ± 33†	10
Adrenalectomy plus THC (3.18 μmoles/kg)	591 ± 48‡	10
Adrenalectomy control plus THC vehicle	516 ± 31§	11
Adrenalectomy control plus THC (0.318 μmole/kg)	706 ± 23§	8

* THC was administered 30 min prior to death.
† $P > 0.05$.
‡ $P > 0.05$.
§ $P < 0.001$.

Table 4. Effect of FLA-63 (50 mg/kg, i.p., 2 hr pretreatment) or α-MT (200 mg/kg, i.p., 4 hr pretreatment) on THC (3.18 μmoles/kg, i.p.)-induced lipolysis in mice

Treatment	Plasma NEFA levels (nmoles/ml)	No. of animals
FLA-63 vehicle plus THC vehicle	589 ± 57*,†	13
FLA-63 vehicle plus THC	826 ± 61†	14
FLA-63 plus THC vehicle	582 ± 51*,‡	15
FLA-63 plus THC	896 ± 69‡	13
α-MT vehicle plus THC vehicle	550 ± 59§,‖	9
α-MT vehicle plus THC	1026 ± 77‖	10
α-MT plus THC vehicle	421 ± 29§,¶	11
α-MT plus THC	549 ± 46¶	9

* $P > 0.05$.
† $P < 0.01$.
‡ $P < 0.01$.
§ $0.1 > P > 0.05$.
‖ $P < 0.001$.
¶ $0.02 < P < 0.05$.

action (Table 5), and in combination with THC, pimozide in spite of its own intrinsic activity produced a partial but non-significant (by *t*-test, Table 5) blockade of the THC response. Using a paired *t*-test, the mean difference ± S. E. M. between (1) the pimozide vehicle plus THC vehicle group and the pimozide vehicle plus THC group and (2) the pimozide plus THC vehicle group and the pimozide group plus THC group was, respectively, 0.296 ± 0.0452 (17 animals) and 0.103 ± 0.034 (17 animals), $t = 3.4106$, $df = 32$, $P = < 0.01$, indicating that there had been a significant inhibition of the THC response by pimozide.

Attention should be drawn to the variation in response to a fixed dose of THC noted in the present series of experiments. For example, 3.18 μmoles THC/kg produced plasma NEFA levels of 749 ± 36, 852 ± 45, 826 ± 61 and 1026 ± 77 nmoles/ml in several experiments. While different drug vehicles could account for some of this variation, we have noted an apparent annual rhythm in the animal's responsiveness to THC, with a decreased response apparently occurring in the southern hemisphere summer. Overall, however, allowing for the fact that the data presented here were collected in experiments conducted over about 9 months, there is a reasonable degree of consistency in the response to THC.

DISCUSSION

We have previously reported [4] that low doses of THC on both oral and i.p. administration produce in mice a rise in plasma NEFA levels, a finding in agreement with an earlier study in rats [2]. We hypothesized at that time that THC could elicit this response either by a direct action on the adipose tissue or indirectly via the release of hypophyseal and/or adrenal cortical hormones.

Adenyl cyclase (and hence lipolysis) in isolated adipocytes is influenced both by stimulation of hormone receptors on the cell membrane [9, 10] and by a negative feedback system exerted by PGE_1 or PGE_2 [11–13]. THC and Δ^8-THC can elicit changes in cAMP levels and in adenyl cyclase and phosphodiesterase activities in certain rat brain areas [14, 15]. Moreover, THC can inhibit PG synthetase *in vitro*

Table 5. Effect of phentolamine (20 mg/kg, i.p., 1 hr pretreatment), perphenazine (0.5 mg/kg, 1 hr), and pimozide (0.5 mg/kg, 2 hr) on THC (3.18 μmoles/kg, i.p.)-induced lipolysis in mice

Treatment	Plasma NEFA levels (nmoles/ml)	No. of animals
Vehicle plus THC vehicle	485 ± 28*,‡	18
Vehicle plus THC	749 ± 36	17
Phentolamine plus THC vehicle	625 ± 37*,†	17
Phentolamine plus THC	800 ± 54†	19
Perphenazine plus THC vehicle	525 ± 47‡,§	8
Perphenazine plus THC	515 ± 43§	9
Pimozide vehicle plus THC vehicle	556 ± 39‖	17
Pimozide vehicle plus THC	852 ± 45	17
Pimozide plus THC vehicle	647 ± 32‖,¶	17
Pimozide plus THC	750 ± 34¶	17

* $P < 0.01$.
† $P < 0.025$.
‡ $P > 0.05$.
§ $P > 0.05$.
‖ $P > 0.05$.
¶ $P < 0.05$.

[16, 17], and can antagonize PGE_1-induced elevation of cAMP in cultured WI-38 fibroblasts [18]. As we have previously shown that THC and PGE_1 interact in an apparently competitive manner *in vivo* [19], we had predicted that THC might alter either basal or stimulated lipolysis in adipocytes by interference with the PGE_2-modulated negative feedback system. In fact, our findings clearly show that neither THC, CBD, 11-OH THC nor 8α,11-diOH THC produced any marked effect *in vitro* on isolated mouse adipocytes. Moreover, none of the five cannabinoids tested altered the response of the adipocytes to ACTH or to isoprenaline. Since several workers (*vide supra*) have demonstrated an interaction between THC and cAMP/PGE_1 systems, and since these systems are intimately involved in lipolysis, the present results cannot at present be satisfactorily explained. The data suggest, however, that the THC-induced lipolysis observed *in vivo* [4, and this paper] is unlikely to be due either to a direct action on the adipose tissue or to a sensitization of the receptors in that tissue to endogenous catecholamines or to ACTH. Other work from this laboratory (Dr. F. Caredes, personal communication) using human platelets *in vitro* as a model system has shown that THC, CBD, CBN, 11-OH THC and 8α,11-diOH THC display no interaction with PGE_1 on platelet aggregation. This finding supports the data reported above. The possibility that THC may require the presence of a glucocorticoid for lipolysis to occur *in vitro* (in a similar manner to the requirement of growth hormone for a glucocorticoid) was eliminated in several experiments where THC (10^{-8} or 10^{-10} M) was incubated with adipocytes in the presence of hydrocortisone 21 acetate (10^{-6} or 10^{-9} M). No significant lipolytic effect was observed. Maier and Maître [2] found that prior hypophysectomy prevented the THC-induced elevation of plasma NEFA in rats. On this basis they suggested that the response was centrally rather than peripherally mediated. We have found that in bilaterally adrenalectomized animals, THC had no effect on plasma NEFA. Although only 24 hr was allowed for recovery after adrenalectomy, the fact that THC was totally ineffective in these animals [see e.g. Refs. 1 and 2] suggests that the corticosteroid levels were depressed to a degree where THC was no longer effective. Since β-adrenergic receptor blockade with propranolol did not prevent the stimulatory effect of THC on plasma NEFA [4], it seems probable that in mice this response involves adrenal cortical hormones. It is perhaps relevant to the latter concept that THC *in vivo* and ACTH *in vivo* produced almost the same maximum elevation of plasma NEFA (although not *in vitro*, *vide supra*) and that these maxima were about 80 per cent of the maximum response to adrenaline. Furthermore, it has been observed in rats that THC cannot only raise the plasma NEFA level, but also that of plasma corticosterone [1, 2], and that prior hypophysectomy prevented these responses. It seems, therefore, that this effect of THC is centrally mediated through the hypophyseal–adrenal cortical axis.

It is well documented that the release of many of the anterior pituitary hormones is modulated indirectly by hypothalamic aminergic pathways. In the present study, it was found that pretreatment with the tyrosine hydroxylase inhibitor α-MT [20] completely blocked the lipolytic response to THC. Since tyrosine hydroxylase is the rate-limiting step in both noradrenaline and DA synthesis, this implies that the effect of THC is dependent upon the newly synthesized stores of either or both of these amines. FLA-63, a potent and fairly specific inhibitor of DA-β-hydroxylase [21], in a dose which has been shown to block this enzyme in mice [22], affected neither the basal NEFA level nor the response to THC, suggesting that an intact noradrenaline synthetic pathway is not essential for the THC-induced lipolytic effect. The involvement of DA in the action of THC was supported by the ability of both pimozide and perphenazine (both potent DA receptor blocking agents) to antagonize the response to THC. It was further found that phentolamine (an α-adrenergic blocking agent in mice, see Dunstan and Jackson [23]), while itself elevating the basal NEFA level, did not block the lipolytic effect of THC. These results suggest that a functional dopaminergic pathway is necessary for this particular response to THC. The most probable pathway would be the tubero-infundibular dopaminergic tract, although an action at a neuronal structure connected to this tract cannot be eliminated. The apparently specific involvement of DA receptors also suggests that THC is not functioning by actually displacing ACTH from the corticotrophs in the anterior pituitary. Our data do not enable us to state whether THC is acting by releasing corticotrophin releasing factor (CRF), although Kubena *et al.* [1] suggested that the increased corticosterone levels in rats produced by THC were dependent on hypothalamic stimulation and on the presence of CRF.

Biochemical studies on the effect of THC on dopaminergic systems in the central nervous system (CNS) have been conflicting. Some studies using rats and mice have found no change in either endogenous DA levels or on DA turnover in both whole brain and in various regions of the brain [24, 25]. In contrast, Maître *et al.* [26] reported that Δ^8-THC stimulated the accumulation of [^{3}H]DA in the corpus striatum and to a lesser extent in the hypothalamus of rats. An increased DA turnover might account at least in part for the increased ACTH secretion produced by THC. It should also be noted that a tyramine-like action for THC has been described in peripheral tissue [27] and, if such an action occurred in the CNS, it would not be in conflict with the data presented here. To clarify these apparently conflicting findings, further biochemical investigations are needed.

The lack of effect of both CBD and CBN on this parameter is particularly interesting, as both of these cannabinoids are comparatively inactive in man. Moreover, the differential activity of THC compared to CBD and CBN suggests perhaps a specific, rather than a non-specific, mechanism of action.

The relevance of the present findings to the psychoactivity of THC cannot be assessed until more is known about the endocrine effects of THC in man. Nevertheless, we feel that the interaction of THC with central dopaminergic mechanisms as presented here warrants further investigation.

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