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PROSTAGLANDINS AND CANNABIS—VI

RELEASE OF ARACHIDONIC ACID FROM HeLa CELLS BY Δ^1-TETRAHYDROCANNABINOL AND OTHER CANNABINOIDS

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Abstract—Treatment of HeLa cells in suspension culture with [^{14}C]arachidonic acid led to a rapid incorporation of this fatty acid into cellular phospholipid pools. Exposure of these labeled cells to Δ^1-tetrahydrocannabinol and other cannabis constituents led to a dose-related release of arachidonic acid into the culture medium. Cannabinol and cannabichromene were also effective, whereas other cannabinoids were less potent and noncannabinoid constituents such as eugenol were without activity. This action of the cannabinoids could have direct effects on cell membrane structure and, in addition, could alter the biosynthesis of prostaglandins and related metabolites of arachidonic acid.

The release of arachidonic acid from phospholipid pools where it is covalently bound is believed to be an important physiological control point in processes mediated by the transformation products of arachidonic acid [1]. Figure 1 depicts these reactions and the subsequent conversion to the prostaglandins (PG) and thromboxanes; the latter stages involving free arachidonic acid probably proceed without direct control, although a number of drugs are thought to modulate the activity of prostaglandin cyclo-oxygenase [2]. The pharmacological diversity of these drugs reflect the wide range of biological mechanisms involving prostaglandins.

In the last few years, we have explored the possibility that certain of the constituents of *Cannabis sativa* exert at least some of the reported actions of cannabis by modulating the levels of PG in target tissues [3–7]. Our initial observations showed that several of the naturally occurring cannabinoids, including Δ^1-tetrahydrocannabinol (THC), gave an apparent inhibition of PGE synthesis in microsomal preparations from ovine or bovine seminal vesicles [3, 4]. A thorough screening of fractions of cannabis extracts also revealed the presence of two potent noncannabinoid inhibitors, eugenol [5] and *p*-vinylphenol [7].

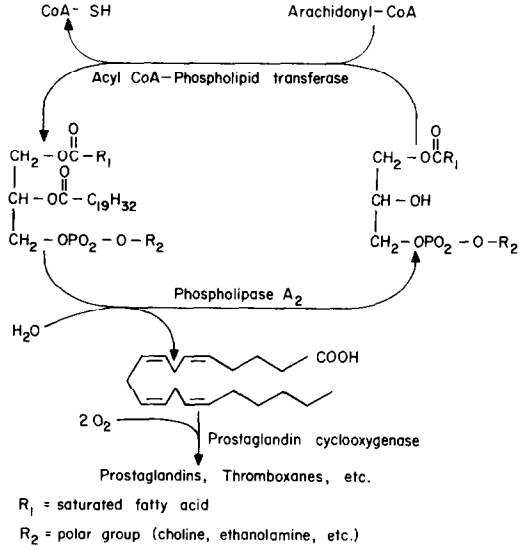

Fig. 1. Initial steps in the biosynthesis of prostaglandins and thromboxanes.

In a continuation of these studies, we examined the effects of several of these agents on mammalian cells in culture which we anticipated would represent a more accurate model of the situation *in vivo*. Using primary monolayer cultures of mouse mammary tumor epithelial cells [8], we discovered a pronounced divergence in the actions of some of the above cannabis constituents. Whereas Δ^1-THC gave a significant stimulation in PG production (Table 1), eugenol essentially abolished PGE synthesis and markedly reduced PGF levels [6]. Cannabinol (CBN), the most active cannabinoid in the microsomal system, showed marginal inhibition in the mammary cell (Table 1). A likely explanation for the stimulatory effect of Δ^1-THC was that it accelerated the release of arachidonic acid from certain phospholipid storage pools in these cells. The purpose of this study is to report data which support the view that the cannabinoids exert some of their pharmacological actions by stimulating the release of arachidonic acid at various target sites.

MATERIALS AND METHODS

Drugs, precursors, etc. Samples of the various cannabinoids were obtained from the National Institute on Drug Abuse and were 93 per cent pure or better. Stock solutions were periodically assayed by thin-layer chromatography (t.l.c.) to monitor decomposition. Olivetol, eugenol, aspirin and *p*-hydroxycinnamic acid were purchased from Aldrich Chemical Co., Milwaukee, WIS.; the unlabeled fatty acids were purchased from Applied Science Labs., State College, PA. [^{14}C]arachidonic acid was obtained from New England Nuclear Corp., Boston, MA and had a specific activity of 50 Ci/mole, *p*-Vinylphenol was prepared by thermal decomposition of *p*-hydroxycinnamic acid as

Table 1. Effects of cannabinoids on prostaglandin production in mouse mammary cells*

	PGF (ng/ml)	PGE (ng/ml)	Total PG (ng/ml)
Control†	3.85 ± 0.40	1.19 ± 0.11	5.04
THC‡ (0.016 μM)	3.27 ± 0.68	0.60 ± 0.10	3.87
THC‡ (0.159 μM)	3.30 ± 0.44	0.77 ± 0.31	4.07
THC‡ (1.59 μM)	8.37 ± 0.12	3.87 ± 0.29	12.24
CBN (0.016 μM)	3.10 ± 0.70	1.60 ± 0.10	4.70
CBN (0.159 μM)	1.73 ± 0.49	0.70 ± 0.10	2.43
CBN (1.59 μM)	2.2 ± 0.10	0.73 ± 0.15	2.93
Control†	2.36 ± 0.23	0.44 ± 0.24	2.80
Eugenol‡ (0.015 μM)	0.33 ± 0.07	0.49 ± 0.02	0.82
Eugenol‡ (0.152 μM)	0.5	<0.2	<0.7
Eugenol‡ (1.52 μM)	1.08	<0.2	<1.28

* Prostaglandin production was estimated by radioimmunoassay. Values are the means ± S.D. of three experiments. Confluent monolayers (day 6) of C3H mouse mammary tumor cells were used. The details of the preparation and culture of these cells are described in Ref. 8.

† Control samples consisted of media plus vehicle (10 μl ethanol).

‡ These values for THC and eugenol were reported at the 1975 Asilomar Conference on "The Therapeutic Potential of Marihuana" (see Ref. 6).

described previously [7]. Phospholipase A_2 (*Trimeresurus flavoviridis*) was purchased from Sigma Chemical Co., St. Louis, MO. The crude venom was heated at 100° for 8 min and the precipitate removed by centrifugation [9].

Preparation of cells. HeLa cells (S_3 strain) were grown in suspension culture at 2.5 to 7 × 10^5 cells/ml in Jokliks. medium (Gibco) containing 3.5% each of calf serum and fetal bovine serum and Kanamycin (10 μg/ml). Mouse mammary epithelial cells were prepared from C3H tumor-bearing animals as described previously [8].

Labeling of cells. HeLa cells (5 × 10^5 cells/ml) were incubated with 75,000 dis./min [^{14}C]arachidonic acid/ml of cell suspension at 37° for 60 min. An aliquot (2 ml) was removed and the cells and supernatant were separated by centrifugation at 1500 *g* for 3 min; the per cent of incorporation was calculated by analyzing the cells and supernatant using procedure B (see below). The remaining labeled cells were centrifuged and the pellets resuspended in Eagle's minimum essential medium (MEM), in the absence of fetal calf serum, equivalent to the original suspension volume.

Drug treatment of the labeled HeLa cells. Labeled cells (2 ml) in 10 ml "Siliclad"-coated Erlenmeyer flasks were incubated at 37° for 60 min with drug delivered in 10 μl ethanol (final concentrations from 0.16 to 160 μM). Controls consisted of 10 μl ethanol added to otherwise identical systems. Suspensions were transferred to tubes and centrifuged and the pelleted cells and supernatants analyzed for radioactivity using either procedure A or B (see below). In a typical experiment, approximately 10^6 cells, containing ^{14}C with 70,000–80,000 dis./min, gave a control value for unstimulated release of about 3000 dis./min.

Siliclad coating of flasks. Clean glassware was immersed for 5 min in 1% Siliclad solution (Clay-Adams) and rinsed thoroughly in water and either allowed to dry overnight at room temperature or at 100° for 10 min.

Analysis of products (procedure A). The incubated HeLa cell suspensions were transferred to tubes and centrifuged at 1500 *g* for 3 min and the arachidonic acid was extracted from the supernatant with 3 ml ether in screw-capped tubes. The incubation flasks were rinsed with 1 ml ether and the combined ether phases applied to silicic acid columns (0.4 g, Unisil, 100–200 mesh. Clarkson Chemical Co., Williamsport, PA) contained in a disposable Pasteur pipette with a glass wool plug [10]. The supernatants were again extracted with 2 ml ether and added to the columns. Eluates collected in scintillation vials were evaporated to dryness under nitrogen and 10 ml of toluene-based scintillation fluid was added and assayed in a Packard Tri-carb liquid scintillation counter. Phospholipids were analyzed by resuspending the cell pellets in 2 ml MEM and extracting with 3 ml $CHCl_3$–MeOH (2:1, v/v), rinsing the incubation flask with 1 ml $CHCl_3$:MeOH and washing the aqueous phase with $CHCl_3$:MeOH (2 ml). The combined organic phases were passed through the same silicic acid columns used above, with a final 1 ml $CHCl_3$:MeOH wash of the column. Eluates were evaporated to dryness and radioactivity was measured. Results were calculated as per cent dis./min ^{14}C released into the supernatant based on total dis./min recovered (channels ratio method was used to determine dis./min). Recoveries of arachidonic acid were 95–100 per cent and phospholipids 65–88 per cent.

Analysis of products (procedure B). The incubated HeLa cell suspensions were centrifuged at 1500 *g* for 3 min. The cells were solubilized in 0.5 ml NCS (Amersham Searle) by warming for 15 min and the tube was subsequently rinsed with 0.5 ml NCS. Ten ml Aquasol (New England Nuclear Corp.) was added to the vial and the sample assayed for radioactivity. Aliquots (2 × 0.5 ml) of the cellular supernatant were added to 10 ml Aquasol and assayed. The per cent incorporation into the cells was calculated as dis./min in the cells/total dis./min recovered × 100.

Quantitative analysis of labeled incorporation and released material. Labeled cells or supernatants were acidified with 1 N H_2SO_4 and extracted with 10 vol.

ethyl acetate resulting in better than 90 per cent recovery of ^{14}C. The extracts were dried with anhydrous Na_2SO_4 and evaporated to dryness under nitrogen and then applied to Silica gel t.l.c. plates (20 × 20 cm, EM Labs, 0.025 cm thick). Chromatography in chloroform–methanol–glacial acetic acid (90:6:6, v/v) was followed by staining with 3.5% phosphomolybdic acid in isopropyl alcohol. Zones were removed and 1 ml ethanol and 10 ml scintillation fluid were added to the vials containing the Silica gel and the radioactivity was measured. Results were calculated as per cent dis./min product based on total dis./min recovered.

Radioimmunoassay of PGE and PGF levels in the mouse mammary cultures was performed by the Herschel Laboratory, Worcester Foundation, as described in a previous publication [8]. The antisera used do not distinguish between the PG_1 and PG_2 series; the extent of crossreaction has been published (see Ref. 8).

RESULTS

Arachidonic acid can be incorporated into phospholipid pools of platelets [11] and cells in culture [12] by simple exposure of the cells to free acid for a suitable period. In the present study, HeLa cells in spinner culture were also found to incorporate [^{14}C]arachidonic acid from the medium (Table 2), providing a model upon which to study the effects of various cannabis components.

The evidence for the nature of this "bound" arachidonic acid was obtained in two ways. First, treatment of the labeled cells with exogenous phospholipase A_2 [9] resulted in the release of substantial radioactivity which was identified as arachidonic acid by t.l.c.; moreover, the isolated labeled phospholipid also was hydrolyzed by phospholipase A_2 to give [^{14}C]arachidonic acid (results to be published). Second, extraction of the cells followed by chromatographic analysis indicated that the bulk of the radioactivity in the cells consisted of phospholipids (Table 3). Less than 2 per cent was free arachidonic acid and somewhat less than 20 per cent was probably a mixture of triglycerides. Further chromatography of the phospholipid fraction by two-dimensional t.l.c. showed that it consisted of one major component with two or three minor phospholipid constituents.

Table 2. Incorporation of [^{14}C]arachidonic acid into HeLa cell phospholipid*

Time (min)	Per cent ^{14}C in media	Per cent ^{14}C in cells
0	93.5	5.2
5	85.5	17.4
10	76.1	24.8
30	54.6	44.9
60	37.9	60.1

* HeLa cells (1.2 × 10^6 cells/2.0 ml) were incubated with [^{14}C]arachidonic acid (150,000 dis./min, 50 mCi/m-mole) at 37° for the indicated time intervals. Values are expressed as the percentage of total radioactivity. Thin-layer chromatography of media indicated mostly unchanged arachidonic acid. Treatment of cells with phospholipase A_2 released about half of the radioactivity as arachidonic acid.

Table 3. Distribution of products from HeLa cells labeled with [^{14}C]arachidonic acid*

Thin-layer chromatography zone†	% Radioactivity	Identity
1 (origin)	66.9	Phospholipids
2	4.6	
3	3.3	PGF
4	1.6	PGE
5	2.8	
6	0.9	PGA
7	1.1	
8	1.9	Arachidonic acid
9	9.6	Triglycerides
10 (front)	7.6	

* HeLa cells (6.7 × 10^5 cells/ml) were incubated with [^{14}C]arachidonic acid for 60 min at 37°.

† Silica gel G was developed in $CHCl_3$–MeOH–HOAc (90:6:6, v/v).

Cells labeled as described above were exposed to a wide range of Δ^1-THC doses for a period of 1 hr. Figure 2 depicts the resulting release of radioactivity at each dose and shows that a marked elevation in the medium occurs between 1.60 and 16.0 μM. Beyond 16.0 μM a less dramatic release of radioactivity occurred. The identity of the released material was established by t.l.c. and consisted almost entirely of arachidonic acid. Sonicated HeLa cells have been reported to synthesize PG [13]; however, the intact cells which we used exhibited a lesser tendency to convert arachidonic acid into PG (Table 4). This may be one factor in explaining the apparently low conversion of labeled arachidonic acid into metabolites which we observed.

The viability of the cells under the conditions of our experiments was ascertained using the Trypan blue exclusion technique. The results are seen in Fig. 2 and indicate that at high doses there is a significant decrease in viability. These doses are, however, beyond those where the release effect occurs.

The effects of the other naturally occurring cannabinoids on arachidonic acid release were of obvious interest, especially in view of their activities in the microsomal preparations [3, 4]. Table 5 summarizes our findings and shows a range of responses up to about a 9-fold stimulation over control values. The most active plant constituents were Δ^1-THC, CBN and cannabichromene, while the most potent metabolite tested was 7-hydroxy-CBN. There is a substantial difference in the potency of Δ^1-THC shown in Table 5 compared to that shown in Fig. 2. This is due to binding of the drug to the walls of the incubation flask in the case of the former. The data in Fig. 2 were obtained using vessels which had been "siliconized" (see Materials and Methods), while those in Table 5 were from untreated glassware.

Table 6 gives the activities of several noncannabinoids; *p*-vinylphenol, *p*-hydroxycinnamic acid and eugenol are components of cannabis, while olivetol, morphine and aspirin were included for comparison purposes. Only olivetol gave some indication of an effect and that was at 160 μM. Both *p*-hydroxycinnamic acid and morphine showed no enhanced release

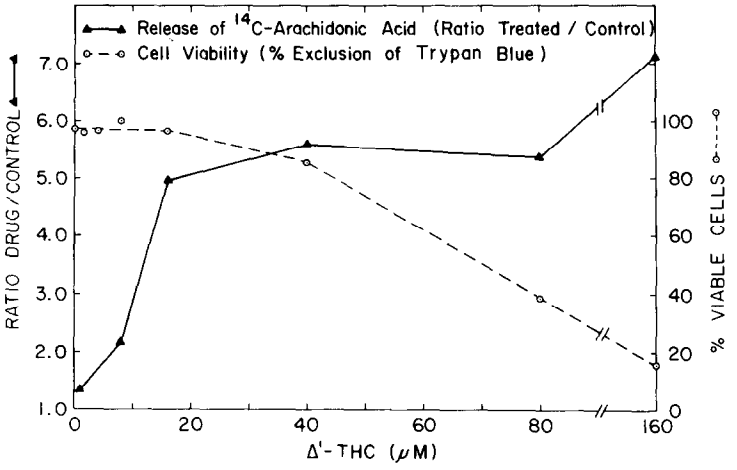

Fig. 2. Effects of Δ^1-THC on HeLa cells.

Table 4. Effect of Δ^1-tetrahydrocannabinol on prostaglandin production in the HeLa cell*

	PGF (ng/ml)	PGE (ng/ml)	Total PG (ng/ml)
Control†	1.09 ± 0.38	1.27 ± 0.55	2.36
THC (0.16 μM)	1.26 ± 0.11	1.55 ± 0.09	2.81
THC (1.6 μM)	1.37 ± 0.33	1.64 ± 0.00	3.01
THC (16 μM)	1.15 ± 0.00	1.91 ± 0.09	3.00
THC (160 μM)	1.42 ± 1.10	6.27 ± 0.55	7.69

* Prostaglandin production was estimated by radioimmunoassay. Values are the means ± S.D. of three experiments. The HeLa cells (6.5×10^5 cells/ml), in log phase of growth, were incubated for 60 min.

† Control consisted of medium (derived from HeLa cells) plus vehicle (10 μl ethanol).

of arachidonic acid, even though in microsomal preparations they stimulate PG synthesis [7, 14].

The question of whether the release effect was confined to arachidonic acid was also examined. Cells were labeled with linoleic, stearic and palmitic acids using the same procedure adopted for arachidonic acid. The effect of Δ^1-THC was then measured and the results are shown in Table 7. There appeared to be a pronounced specificity for arachidonic acid release; decreasing the degree of unsaturation and/or shortening the chain length caused a diminished response to the stimulatory effects of the drug.

Table 5. Release of arachidonic acid by cannabinoids*

	Cannabinoid concentration (μM)			
	0.160	1.60	16.0	160
Δ^1-THC	0.970	1.09	2.63	8.78
CBN	1.40	1.34	2.32	8.43
6α-OH-Δ^1-THC	1.11	1.24	1.97	4.63
6β-OH-Δ^1-THC	1.19	1.26	1.84	4.49
Cannabidiol	1.65	1.53	2.72	4.86
7-OH-CBN	1.09	1.09	2.40	8.55
Cannabicyclol	1.15	1.33	1.29	1.87
Cannabichromene	1.15	1.27	1.69	9.39
5-″OH-Δ^1-THC	1.13	1.09	1.22	2.86
1-COOH-7-nor-CBN		1.16	1.46	2.36

* HeLa cells were prelabeled with [^{14}C]arachidonic acid followed by drug treatment for 60 min at the indicated concentrations in unsiliconized glassware. The data are given as the ratios of released ^{14}C from treated and untreated cells and are the averages of duplicate experiments.

DISCUSSION

The results presented in this report demonstrate that Δ^1-THC and several other cannabinoids can cause a turnover or depletion of phospholipid bound arachidonic acid in a dose-related fashion in the HeLa cell. Similar effects in other systems have been reported with substances such as bradykinin [12], thyroid-stimulating hormone [15] and thrombin [11, 12]. A release of arachidonic acid has also been

Table 6. Release of arachidonic acid by noncannabinoids*

	Drug concentration (μM)		
	1.60	16.0	160
p-Vinylphenol	1.22	1.02	1.17
p-OH-cinnamic acid	1.22	1.07	0.965
Olivetol	1.00	0.965	1.71
Eugenol	1.04	1.18	1.52
Morphine	1.07	0.928	1.15

* HeLa cells were prelabeled with [^{14}C]arachidonic acid followed by drug treatment for 60 min at the indicated concentrations. The data are given as the ratios of released ^{14}C from treated and untreated cells and are the averages of duplicate experiments.

Table 7. Fatty acid specificity*

	THC concentration (μM)		
	1.60	16.0	160
Arachidonic acid (20:4)	1.30	2.40	6.35
Linoleic acid (18:2)	0.96	1.14	1.60
Stearic acid (18:0)	1.02	1.26	1.33
Palmitic acid (16:0)	0.92	0.96	0.97

* HeLa cells were prelabeled with ^{14}C-fatty acid followed by drug treatment for 60 min at the indicated concentrations in unsiliconized glassware. The data are given as the ratio of released ^{14}C from treated and untreated cells and are the averages of duplicate experiments.

evoked by mechanical vibration or immunological challenge of guinea pig spleen slices [1]. While the overall effects of the cannabinoids are similar to these agents, in their respective systems it seems likely that they act at different sites.

Substances such as bradykinin undoubtedly have specific receptors to which they bind resulting in a stimulation of either phospholipase A_2 or some mediator such as adenyl cyclase. No high affinity receptor has yet been identified for Δ^1-THC; however, it does "dissolve" readily in membranes [16] and because of its lipophylic nature would probably concentrate in the hydrophobic regions of target membranes. There may be structural requirements for a particular cannabinoid to enter into these regions which is perhaps analogous to that for membrane-bound sterols [17].

Cannabinoids are known to cause physical perturbations of both natural [18] and synthetic membranes [19] and these may manifest themselves by modulating effects on nearby membrane-bound enzyme systems. In the present report, an apparent activation of phospholipase A_2 has been observed. In addition, there are reports that other membrane-associated systems involving Mg^{2+} and Na^+-K^+ ATPase, acetylcholinesterase [20, 21], glutamine synthetase [21] and adenylate cyclase [22], also respond to Δ^1-THC.

Another class of drugs, namely the local anesthetics, cause changes in phospholipase activities [23]. It might be expected, then, that some interaction would be observed between the cannabinoids and these anesthetics. Although this has not yet been reported, synergistic actions of Δ^1-THC with certain anesthetics have, in fact, been observed [24]. It is interesting to note that, in a discussion of the general pharmacology of the cannabinoids, Paton *et al.* [25] have likened Δ^1-THC to a "partial anesthetic."

The noncannabinoid substances listed in Table 6 showed essentially no activity in stimulating the release of arachidonic acid. One such substance, eugenol, was also tested for its effect on endogenous PG production (Table 1) in the mammary cell where it caused a lowering at several different doses. It seems, then, that these compounds react only with the prostaglandin cyclo-oxygenase system. On structural grounds it would be expected that they should be pharmacologically different than the companion cannabinoid constituents of marihuana.

In most systems thus far reported on, the release of arachidonic acid is followed by an increase in prostaglandin synthesis. In the HeLa cell, under our conditions, we did not see any significant increase in PG using the labeled precursor method of analysis. Stimulation of prostaglandin synthesis was seen in the mouse mammary cell where the production of PG from endogenous arachidonic acid was monitored by radioimmunoassay (Table 1). A likely explanation for this difference is that in the labeled precursor method there is a large dilution of [^{14}C]arachidonic acid by released, endogenous, unlabeled acid which would obscure the relatively small conversions to the prostaglandins. This is supported to an extent by the results in Table 4 which show that HeLa cells have somewhat elevated prostaglandin levels in the presence of Δ^1-THC.

The present findings on the release of prostaglandin precursor by Δ^1-THC need to be accommodated with our previous reports that the latter is an inhibitor of prostaglandin cyclo-oxygenase. There are, of course, obvious differences in the model system. The limited data in Table 1, however, suggest a dose-related explanation, namely while PGF and total prostaglandin production were increased at all Δ^1-THC doses, PGE levels were decreased at the lower drug levels. In our previous studies [3, 4] using the microsomal system, only PGE synthesis was measured.

CONCLUSIONS

First, treatment of HeLa cells with Δ^1-THC, the principal psychoactive constituent of cannabis, causes a profound release of phospholipid-bound arachidonic acid. The effective dose range is between 1.6 and 16 μM.

Second, the above doses of Δ^1-THC do not produce changes in cell viability as measured by dye exclusion; however, drug levels beyond 40 μM result in significant decreases in viability. It is not clear whether the decrease in viability is a result of excessive depletion of membrane arachidonic acid or some other effect possibly mediated by the release of arachidonic acid.

Third, other cannabinoids such as CBN and cannabichromene also stimulate the release of arachidonic acid, while cannabicyclol shows little activity. A correlation of this effect on the HeLa cell with the known pharmacological activities of the various cannabinoids is difficult because of the numerous biological processes affected by arachidonic acid-dependent reactions. This effect may, however, reflect the diverse actions of Δ^1-THC and other cannabinoids.

Fourth, the stimulation of PG production by high levels of Δ^1-THC in mouse mammary epithelial cells, in the isolated perfused guinea pig lung [26] and in rat uterine venous blood [27] is probably due to an increase in arachidonic acid precursor. Lower doses of Δ^1-THC may cause inhibition of prostaglandin synthesis, as is suggested by our findings in the mammary cell.

Finally, this action of Δ^1-THC on the HeLa cell is highly dependent on the structure of the fatty acid suggesting a specific action on a phospholipase A_2 system.

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REFERENCES

1. R. J. Flower and G. J. Blackwell, *Biochem. Pharmac.* **25**, 285 (1976).
2. H. J. Robinson and J. R. Vane (Eds), *Prostaglandin Synthetase Inhibitors*, pp. 19–52. Raven Press, New York (1974).
3. S. Burstein and A. Raz, *Prostaglandins* **2**, 369 (1972).
4. S. Burstein, E. Levin and C. Varanelli, *Biochem. Pharmac.* **22**, 2905 (1973).
5. S. Burstein, C. Varanelli and L. T. Slade, *Biochem. Pharmac.* **24**, 1053 (1975).
6. S. Burstein, in *The Therapeutic Potential of Marihuana* (Eds S. Cohen and R. C. Stillman), Chap. 2. Plenum, New York (1975).
7. S. Burstein, P. Taylor, F. S. El-Feraly and C. Turner, *Biochem. Pharmac.* **25**, 2003 (1976).
8. S. Burstein, G. Gagnon, S. A. Hunter and D. V. Maudsley, *Prostaglandins* **13**, 41 (1977).
9. Y. Imai and R. Sato, *Biochim. biophys. Acta* **42**, 164 (1960).
10. D. S. Goodman and T. Shiratori, *J. Lipid Res.* **6**, 307 (1965).
11. T. K. Bills, J. B. Smith and M. J. Silver, *Biochim. biophys. Acta* **424**, 303 (1976).
12. S. L. Hong and L. Levine, *J. biol. Chem.* **251**, 5814 (1976).
13. F. Cohen and B. M. Jaffe, *Biochem. biophys. Res. Commun.* **55**, 724 (1973).
14. H. O. J. Collier, W. J. McDonald-Gibson and S. A. Saeed, *Br. J. Pharmac.* **58**, 193 (1976).
15. B. Haye, S. Champion and C. Jacquemin, *Fedn Eur. Biochem. Soc. Lett.* **30**, 253 (1973).
16. P. Seeman, M. Chaow-Wong and S. Mayen, *Can. J. Physiol. Pharmac.* **50**, 1193 (1972).
17. M. K. Jain, in *Current Topics in Biomembranes and Related Systems* (Eds. F. Bronner and A. Kleinzeller), Vol. 6, p. 1. Academic Press (1975).
18. A. Chari-Bitron, in *Marihuana, Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), Chap. 24. Springer, New York (1976).
19. E. W. Gill and D. K. Lawrence, in *The Pharmacology of Marihuana* (Eds M. C. Braude and S. Szara), p. 147. Raven Press, New York (1976).
20. B. Laurent and P. E. Roy, *Int. J. clin. Pharmac.* **12**, 261 (1975).
21. M. K. Poddar and J. J. Ghosh, in *The Pharmacology of Marihuana* (Eds M. C. Braude and S. Szara), p. 157. Raven Press, New York (1976).
22. L. A. Kelly and R. W. Butcher, *Biochim. biophys. Acta* **320**, 540 (1973).
23. G. Scherphof and H. Westenberg, *Biochim. biophys. Acta* **398**, 442 (1975).
24. *Marihuana and Health, Fourth Report to the U.S. Congress*, p. 136. U.S. Govt. Printing Office, Washington, D.C. (1974).
25. W. D. M. Paton, R. G. Pertwee and D. Temple, in *Cannabis and Its Derivatives* (Eds W. D. M. Paton and J. Crown), p. 72. Oxford University Press, London (1972).
26. S. Kaymakcalan, Z. S. Ercan and R. K. Türker, *J. Pharm. Pharmac.* **27**, 564 (1975).
27. V. C. Jordan and V. D. Castracane, *Prostaglandins* **12**, 1073 (1976).