Biochemical Pharmacology, Vol. 31, No. 14, pp. 2361–2365, 1982.
Printed in Great Britain.

0006-2952/82/142361-05 $03.00/0

PROSTAGLANDINS AND CANNABIS—IX

STIMULATION OF PROSTAGLANDIN E_2 SYNTHESIS IN HUMAN LUNG FIBROBLASTS BY Δ^1-TETRAHYDROCANNABINOL

SUMNER BURSTEIN,* SHEILA A. HUNTER, CAROLYN SEDOR and STEVEN SHULMAN
Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

(*Received* 2 *October* 1981; *accepted* 23 *December* 1981)

Abstract—Preliminary data [S. Burstein and S. A. Hunter, *Biochem. Pharmac.* **27**, 1275 (1978)] showed that cannabinoids at levels of 1 μM or greater elevated the concentrations of prostaglandins in cell culture models. Further study [S. Burstein and S. A. Hunter, *J. clin. Pharmac.* **21**, 240S (1981)] led to the suggestion that this effect was due to a stimulation of phospholipase A_2 resulting in the release of free arachidonic acid which was then partly converted into the prostaglandin(s) normally synthesized by the particular target system. The present report gives detailed data on the cannabinoid-induced synthesis of prostaglandin E_2 by the WI-38 fibroblast derived from human lung. The effect could be blocked by pretreatment with mepacrine, a phospholipase inhibitor, and aspirin, a cyclooxygenase inhibitor. These findings lend support to the hypothesis that some of the *in vivo* actions of the cannabinoids are due to modulations in prostaglandin synthesis at various tissue sites.

The mechanism of action of the cannabinoids at the molecular level remains highly speculative despite an abundant literature describing their effects in a wide variety of systems. We have recently reviewed the biochemistry of the cannabinoids and suggested hypotheses on the molecular events resulting from their interactions with various tissue processes [1]. We theorized that the "low-dose" acute responses of the cannabinoids are due to their biotransformation to alkaloid-like metabolites which produce effects such as changes in gonadotrophin secretion, hypothermia and mood alteration. On the other hand, the "high-dose" chronic effects, which seem to involve membrane-bound enzymes such as monoamine oxidase, adenosine triphosphatase and phospholipase A_2 (PLA_2), are explained by a binding of the cannabinoids to sterol binding sites in membranes in close proximity to these enzymes. The resulting perturbation of the lipid environment would cause changes in the activities of these regulatory systems.

In the case of PLA_2, the presence of cannabinoids produces an enhancement of the hydrolytic activity of this enzyme [2, 3]. The stimulation of PLA_2 activity was observed both in isolated whole cell systems and in subcellular fractions derived from these cells. Three diverse cell types were studied, HeLa cells, Leydig cells and WI-38 lung fibroblasts, and all three gave similar results. A significant elevation of enzyme activity was seen with concentrations of Δ^1-tetrahydrocannabinol (Δ^1-THC) starting around 3.2 μM. Moreover, the stimulatory effect of the drug could be antagonized by PLA_2 inhibitors such as mepacrine. A similar observation on the stimulatory action of Δ^1-THC has been made recently using human platelets [4].

An important consequence of the cannabinoid stimulation of PLA_2 is an increase in the production of prostaglandins (PG) in various experimental models. Δ^1-THC caused the release of "PG-like" material from perfused rabbit kidney and guinea pig lung [5] and a rise of PGE in ovarian venous blood from drug-treated rats [6]. In a preliminary study, we have recently detected an increase in canine arterial prostacyclin levels following the i.v. administration of 0.1 to 0.5 mg/kg of Δ^1-THC which was concurrent with the fall in mean arterial blood pressure (unpublished observation). This THC-induced decrease in blood pressure could be prevented by prior administration of aspirin [7]. Dietary depletion of arachidonic acid in mice caused a reduction in their cataleptic response to Δ^1-THC [8] suggesting that prostaglandins were required for the full effect. Several experiments using cultured HeLa cells also indicated a positive effect on prostaglandin production by Δ^1-THC [2]. The present report is a further study of the stimulatory effects on PGE_2 production by Δ^1-THC, cannabinol (CBN) and cannabidiol (CBD), three of the major cannabinoids present in marihuana. The WI-38 human lung fibroblast was chosen as our model both for its convenience in the laboratory and its possible relevance to a major site of cannabinoid tissue uptake.

MATERIALS AND METHODS

Chemicals. Quinacrine (mepacrine) dihydrochloride and bovine serum albumin, fraction V (BSA), were purchased from the Sigma Chemical Co., St. Louis, MO; aspirin was obtained from the Aldrich Chemical Co., Milwaukee, WI. [1-^{14}C]Arachidonic acid (sp. act. 52.7 mCi/mmole) and [^{3}H]PGE_2 (sp. act. 165 Ci/mmole) were purchased from the New England Nuclear Corp., Boston, MA, and purified by thin-layer chromatography. PGE_2 was a gift from the Upjohn Co., Kalamazoo, MI.

* Author to whom correspondence should be addressed.

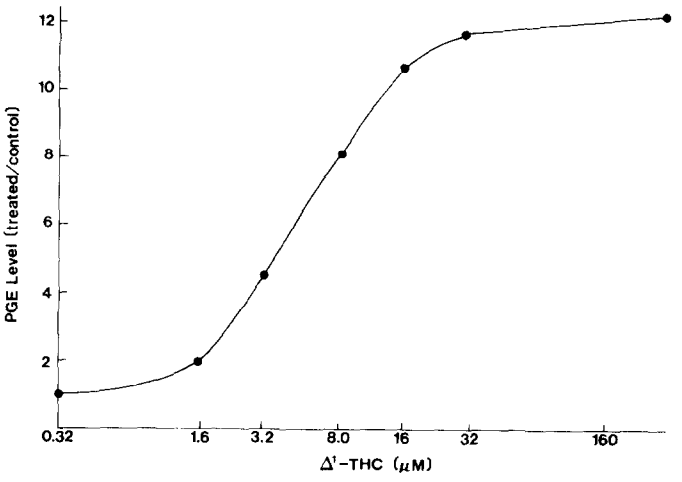

Fig. 1. Effect of increasing concentrations of Δ^1-THC on PGE_2 levels in monolayer cultures of WI-38 human lung fibroblasts. The cells were grown and treated as described in Materials and Methods. Each data point is the mean of six determinations. The stimulated cells were significantly different from controls at the $P < 0.0005$ level using Student's t-test ($N = 6$) at each dose level tested.

The cannabinoids were supplied by the National Institute on Drug Abuse, and their purity was monitored by gas chromatography and found to be greater than 95% pure. PGE-antiserum was donated by Dr. R. Skarnes, Worcester Foundation, Shrewsbury, MA. The cross-reactivities with other prostaglandins were: $PGF_{1\alpha}$, 4.0%; 6-keto-$PGF_{1\alpha}$, 1.0%; and PGD_2, <0.7%. Falcon plastic ware was used throughout the radioimmunoassay (RIA) procedures. NCS tissue solubilizer was purchased from Amersham, Arlington Heights, IL.

Cells. Human lung WI-38 fibroblast cells were obtained from Dr. L. Kelly and had been seeded from stock originally obtained from Dr. L. Hayflick. The cells were grown to confluence (3.1×10^5 cells/35 mm dish) in minimum essential medium (MEM) containing 10% fetal calf serum as described previously [9].

The pattern of prostaglandin production in this cell type has been elucidated by mass spectrometric studies [10]. These authors showed that PGE_2 is a major product formed as a result of cyclooxygenase action and that the only other measurable prostaglandin was thromboxane B_2. Earlier findings from the same laboratory showed that arachidonic acid was present in five times the amount of dihomo-gamma-linoleic acid, suggesting that the PG_2 series is the dominant one in this cell type [11].

Incubation with cannabinoids and inhibitors. WI-38 cells were washed three times with MEM to eliminate serum. Then 1 ml MEM (pH 7.4), containing 0.1% BSA, was added to the cells and preincubated for 30 min at 37° in an atmosphere of 95% O_2:5% CO_2 with inhibitor or its vehicle (10 μl water, adjusted to pH 7.4 with $NaHCO_3$). Cannabinoid or vehicle (10 μl ethanol) was then incubated with the cells for 60 min. The media from the cultures were harvested and centrifuged at 2000 g for 10 min, and the supernatant fractions were stored in polypropylene tubes at −15°; subsequently, the PGE_2 was determined by RIA using a procedure described by Seragen Inc., Boston, MA. Briefly, this involved adding the antibody to a gelatin–Tris buffer solution of the [^{3}H]PGE_2 and a 0.1 ml aliquot of the cell culture medium. After incubating for 1.5 hr at 20°, the unbound PGE_2 was removed by treatment with dextran-coated charcoal at 4°. Aliquots of the bound fraction were assayed for tritium by liquid scintillation counting, and the percent bound was compared with known standards. Each sample was assayed in duplicate. The results were expressed as ng PGE_2/ml.

Labeling of cells. The WI-38 cells (approximately 3×10^5 cells/dish) were incubated with [1-^{14}C]arachidonic acid (650,000 dpm) in 1 ml of serum free-MEM for 60 min. The labeled cells were washed free of unreacted fatty acid with MEM. Under these

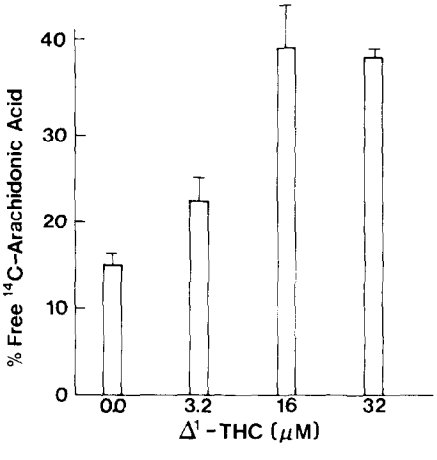

Fig. 2. Effect of increasing concentrations of Δ^1-THC on the release of [^{14}C]arachidonic acid from labeled WI-38 human lung fibroblasts. The cells were grown and treated as described in Materials and Methods. Each value is the mean of triplicate determinations; the bars indicate the standard error of the mean. The stimulated cells were significantly different from controls at the $P < 0.05$ level using Student's t-test ($N = 3$) at each dose level tested.

Table 1. Stimulation of prostaglandin E_2 production in WI-38 fibroblasts by Δ^1-THC*

Cannabinoid	Inhibitor	PGE_2 (ng/ml) ± S.D.†	% Inhibition
		0.53 ± 0.45	
	ASA (56 μM)	<0.1	
Δ^1-THC (3.2 μM		14.5 ± 1.80	
Δ^1-THC (3.2 μM)	ASA (0.56 μM)	14.5 ± 3.01	
Δ^1-THC (3.2 μM)	ASA (5.6 μM)	3.27 ± 0.40	77.0
Δ^1-THC (3.2 μM)	ASA (56 μM)	<0.1	100
Δ^1-THC (16 μM)		50.7 ± 3.21	
Δ^1-THC (16 μM)	ASA (0.56 μM)	47.0 ± 4.09	7.0
Δ^1-THC (16 μM)	ASA (5.6 μM)	13.2 ± 1.53	74.0
Δ^1-THC (16 μM)	ASA (56 μM)	<0.1	100
Δ^1-THC (3.2 μM)	Mepacrine (0.2 μM)	15.4 ± 3.56	
Δ^1-THC (3.2 μM)	Mepacrine (2.0 μM)	14.1 ± 1.72	2.60
Δ^1-THC (3.2 μM)	Mepacrine (20 μM)	0.80 ± 0.76	94.5
Δ^1-THC (16 μM)	Mepacrine (0.2 μM)	38.5 ± 4.50	24.0
Δ^1-THC (16 μM)	Mepacrine (2.0 μM)	21.7 ± 2.55	57.2
Δ^1-THC (16 μM)	Mepacrine (20 μM)	1.07 ± 0.45	97.9

* Approximately 3×10^5 cells were treated with inhibitor (or vehicle) for 30 min at 37°; the cannabinoid (or vehicle) was then added, and the incubation was continued for 60 min. Post-treatment viabilities of the cells ranged from 80 to 100% as determined by measurement of cellular DNA.

† Prostaglandin E_2 concentrations were measured by radioimmunoassay as described in Materials and Methods. N = 3.

conditions, greater than 35% of the incorporated radioactivity was present as phospholipids [3].

Drug treatment of the labeled cells. Labeled cells were incubated at 37° for 60 min with cannabinoid delivered in 10 μl ethanol and added to 1 ml MEM containing 0.1% BSA. The supernatant fraction was separated and analyzed for radioactivity. Chromatographic studies previously reported by us [3] showed that the released radioactivity consisted almost entirely of arachidonic acid. The cells were scraped from the dish using MEM; the suspension was then centrifuged at 2000 *g* for 5 min. The cell pellet was solubilized in 2 ml NCS at 40° for 10 min. All samples were assayed in 10 ml of 947-scintillation fluid (NEN) using a Packard Tri-Carb liquid scintillation spectrometer. The data were expressed as percent dpm product released based on total dpm recovered. Post-treatment cell monolayer survival was determined by measuring cellular DNA and ranged from 80 to 100%.

RESULTS

The addition of Δ^1-THC to monolayers of WI-38 fibroblasts has profound effects on the production of PGE_2 by these cells (Fig. 1). At a concentration

Table 2. Stimulation of prostaglandin E_2 production in WI-38 fibroblasts by cannabinol*

Cannabinoid	Inhibitor	PGE_2 (ng/ml) ± S.D.†	% Inhibition
		0.35 ± 0.45	
CBN (3.2 μM)		3.27 ± 0.51	
CBN (3.2 μM)	ASA (2.8 μM)	3.17 ± 0.68	3.1
CBN (3.2 μM)	ASA (5.6 μM)	<0.1	100
CBN (3.2 μM)	ASA (28 μM)	<0.1	100
CBN (3.2 μM)	Mep (2.0 μM)	0.92 ± 0.39	72
CBN (3.2 μM)	Mep (20 μM)	<0.1	100
CBN (16 μM)		14.0 ± 1.36	
CBN (16 μM)	ASA (2.8 μM)	12.7 ± 5.84	9.6
CBN (16 μM)	ASA (5.6 μM)	3.31 ± 2.29	76
CBN (16 μM)	ASA (28 μM)	<0.1	100
CBN (16 μM)	Mep (2.0 μM)	6.48 ± 2.27	54
CBN (16 μM)	Mep (20 μM)	<0.1	100
	ASA (28 μM)	<0.1	
	Mep (20 μM)	<0.1	

* See the legend of Table 1 for experimental details.

† Prostaglandin E_2 concentrations were measured by radioimmunoassay as described in Materials and Methods. N = 3.

Table 3. Stimulation of prostaglandin E_2 production in WI-38 fibroblasts by cannabidiol*

Cannabinoid	Inhibitor	PGE_2 (ng/ml) ± S.D.†	% Inhibition
		0.30 ± 0.26	
	ASA (5.6 μM)	0.17 ± 0.29	
CBD (3.2 μM)		4.40 ± 0.53	
CBD (3.2 μM)	ASA (0.56 μM)	2.83 ± 0.59	36.7
CBD (3.2 μM)	ASA (2.8 μM)	0.60 ± 0.56	86.4
CBD (3.2 μM)	ASA (5.6 μM)	<0.1	100
CBD (3.2 μM)	ASA (28 μM)	<0.1	100
CBD (16 μM)		49.2 ± 4.19	
CBD (16 μM)	ASA (0.56 μM)	28.5 ± 6.06	42.0
CBD (16 μM)	ASA (2.8 μM)	6.80 ± 0.56	86.2
CBD (16 μM)	ASA (5.6 μM)	0.57 ± 0.21	98.8
CBD (16 μM)	ASA (28 μM)	<0.1	100

* See the legend of Table 1 for experimental details.

† Prostaglandin E_2 concentrations were measured by radioimmunoassay as described in Materials and Methods. N = 3.

of 1.6 μM the drug caused a highly significant 88% stimulation of PGE_2 synthesis. The effect reached a maximum at around 16 μM where a 12-fold stimulation was observed. In other experiments we found a parallel, though less dramatic, increase in the release of free arachidonic acid from WI-38 monolayers (Fig. 2). The dose–response effect was similar especially in terms of maximum stimulation in each case suggesting an experimental as well as a theoretical relationship between the level of released arachidonic acid and PGE_2.

The effects of various antagonists of prostaglandin synthesis were also tested to see if the THC-induced stimulation could be blocked. Table 1 shows the results obtained with aspirin (ASA) and mepacrine, well established inhibitors of arachidonic metabolism and release respectively [12, 13]. Both drugs were quite effective in antagonizing the stimulatory action of Δ^1-THC.

Other cannabinoids were tested for agonist actions in this particular experimental system. Table 2 shows that cannabinol was also effective in elevating PGE_2 levels and that this could be blocked by the prior addition of aspirin or mepacrine. Cannabidiol, an abundant constituent of cannabis, was similarly effective in stimulating PGE_2 synthesis as seen from the data in Table 3. Aspirin again proved to be a potent antagonist as with the previous two cannabinoids.

DISCUSSION

An earlier report from our laboratory gave limited data which indicated that cannabinoids could stimulate the production of prostaglandins by cells in culture [2]. The results presented in this report confirm our previous findings and give a more complete picture of the nature of this effect.

The most likely site for such a stimulatory action is the PLA_2 or other lipase which controls precursor availability for prostaglandin synthesis in a wide variety of systems [13]. We have, in fact, published evidence which suggests a direct action of the cannabinoids on this key regulatory enzyme [3]. In this report we have shown a correlation between the cannabinoid-sensitive release of arachidonic acid from WI-38 cells and the production of PGE_2 under identical experimental conditions. A comparison of the data in Figs. 1 and 2 shows that both effects occur over a similar dose range.

The magnitude of the maximum response on a relative basis is somewhat different in each case, the PGE_2 synthesis effect being greater. We have observed that less than 1% of the released [^{14}C]arachidonic acid is converted to PGE_2 (unpublished data) so that a small increase in free arachidonic acid could readily account for a larger change in prostaglandin levels on a percentage basis. In absolute terms, the PGE_2 levels were in ng/ml (Tables 1–3) whereas free arachidonic acid is present at μg/ml levels [10]. Thus, it seems reasonable that the cannabinoid-induced activity of PLA_2 and the elevated PGE_2 synthesis may be related events.

More evidence that the site of cannabinoid action is at the precursor availability level comes from the data obtained with blocking agents (Tables 1–3). Aspirin is a prostaglandin synthetase inhibitor *in vitro* as well as *in vivo* [12]. In our experimental model, aspirin caused a dose-related decrease in the cannabinoid stimulation of PGE_2 synthesis as expected. In addition, mepacrine, an established PLA_2 inhibitor, also prevented the cannabinoid effect. Mepacrine has little effect on prostaglandin synthetase, suggesting that the site of cannabinoid action is probably PLA_2. Although cannabinoid stimulation of cyclooxygenase cannot be ruled out completely, this seems unlikely in view of our earlier findings which showed inhibition of PGE_2 synthesis in a microsomal preparation of prostaglandin synthetase [14, 15].

The pharmacological significance of these findings has already been discussed above. The possible molecular consequences of this effect, however, are worth some comment at this point. A likely sequel to the elevation of PGE_2 in the WI-38 fibroblast would be the stimulation of adenylate cyclase. It has been amply demonstrated that the addition of PGE_1 causes a dramatic increase in cAMP levels in these cells [9]. We would expect, therefore, on the basis of our present report that cannabinoids should pro-

duce a similar change in cAMP levels. This, in fact, has been reported to occur under conditions not very different from those employed by us in this publication [16]. It seems, therefore, that the "prostaglandin hypothesis" for a mode of action of the cannabinoids [1] has a firm biochemical basis.

Acknowledgements—This work was supported by Research Grants (RO1 DA02043 and RO1 DA02052) and a Research Scientist Award (KO5 DA0043) from the National Institute on Drug Abuse. We thank John Cruz for help in growing the cells for this project.

REFERENCES

1. S. Burstein and S. A. Hunter, *Rev. pure appl. pharmac. Sci.* **2**, 155 (1981).
2. S. Burstein and S. A. Hunter, *Biochem. Pharmac.* **27**, 1275 (1978).
3. S. Burstein and S. A. Hunter, *J. clin. Pharmac.* **21**, 240S (1981).
4. H. L. White and R. L. Tansik, *Prost. Med.* **4**, 409 (1980).
5. S. Kaymakcalan, Z. S. Ercan and R. K. Turker, *J. Pharm. Pharmac.* **27**, 564 (1975).
6. V. C. Jordan and V. D. Castracane, *Prostaglandins* **12**, 1073 (1976).
7. S. Burstein, K. Ozman, E. Burstein, N. Palermo and E. Smith, *Biochem. Pharmac.* **31**, 591 (1982).
8. J. W. Fairbairn and J. T. Pickens, *Br. J. Pharmac.* **69**, 491 (1980).
9. L. A. Kelly and R. W. Butcher, *J. biol. Chem.* **249**, 3098 (1974).
10. R. W. Bryant, S. J. Feinmark, H. J. Makheja and J. M. Bailey, *J. biol. Chem.* **253**, 8134 (1978).
11. L. M. Dunbar and J. M. Bailey, *J. biol. Chem.* **250**, 1152 (1975).
12. R. J. Flower and J. R. Vane, in *Prostaglandin Synthetase Inhibitors* (Eds. H. J. Robinson and J. R. Vane), p. 9. Raven Press, New York (1974).
13. G. J. Blackwell, R. J. Flower, F. P. Nijkamp and J. R. Vane, *Br. J. Pharmac.* **62**, 79 (1978).
14. S. Burstein and A. Raz, *Prostglandins* **2**, 369 (1972).
15. S. Burstein, E. Levin and C. Varanelli, *Biochem. Pharmac.* **22**, 2905 (1973).
16. L. A. Kelly and R. W. Butcher, *J. Cyclic Nucleotide Res.* **5**, 303 (1979).