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PROSTAGLANDINS AND CANNABIS—II

INHIBITION OF BIOSYNTHESIS BY THE NATURALLY OCCURRING CANNABINOIDS*

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Abstract—A series of the naturally occurring cannabinoids were tested for possible effects on the biosynthesis of prostaglandins. Most of the substances examined were able to inhibit in varying degrees the conversion of 8,11,14-eicosatrienoic acid to prostaglandin E_1 (PGE_1) when incubated with bovine seminal vesicle microsomes. The order of activity starting with the most potent was cannabinol, cannabidiolic acid, Δ^6-tetrahydrocannabinol (Δ^6-THC), cannabidiol, cannabichromene and Δ^1-THC; cannabicyclol showed almost no inhibitory activity. It is suggested that certain of the pharmacological actions of some of these cannabinoids may be explained by a similar effect *in vivo*. Olivetol, which represents a partial structure for all of the compounds tested, showed high activity, indicating that the inhibitory power of the cannabinoids resides in the aromatic portion of the molecule.

IN THE FIRST report of this series,[1] we gave evidence that Δ^1-tetrahydrocannabinol† (Δ^1-THC), the major psychoactive component of cannabis, could inhibit the synthesis of prostaglandin E_2 (PGE_2). The data were obtained by measuring the conversion of ^{14}C-arachidonic acid to PGE_2 in the presence of sheep seminal vesicle microsomes under well-established conditions.[2] The inhibitory effect of Δ^1-THC was studied over a limited range and found to be proportional to its concentration. By extrapolation of the results a dose giving 50 per cent inhibition (ID_{50}) of approximately 80–100 μg/ml was observed.

Cannabis contains a number of substances which are chemically related[3] (Fig. 1); however, only two, Δ^1-THC and Δ^6-THC, are believed to be psychoactive.[4] Crude extracts of the plant seem to exert slightly different action than pure THC, suggesting that there are other pharmacologically active substances present.[5] For example, cannabidiol (Fig. 1) has been shown to modify the action of Δ^1-THC,[6] and there have been some reports of biological activity for other cannabis components.[7] It was, therefore, of interest to test whether other cannabinoids could inhibit PG synthesis and whether there was any correlation between psychoactive potency and inhibitory power. The following report describes some experiments we carried out which were designed to answer this question.

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† List of abbreviations: THC = tetrahydrocannabinol; CBN = cannabinol; CBD = cannabidiol; CBD acid = cannabidiolic acid; CBC = cannabichromene; CBCy = cannabicyclol; PG = prostaglandin; LSD = lysergic acid diethylamide; BSV = bull seminal vesicles.

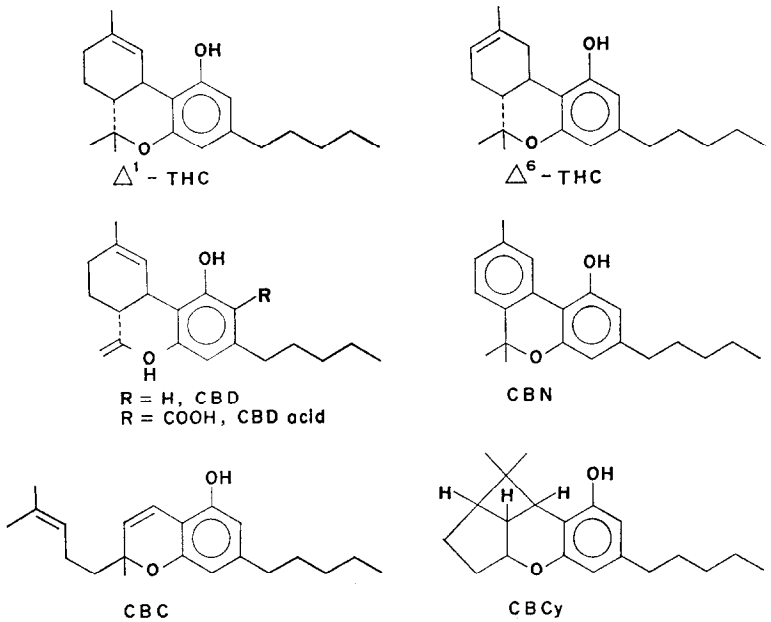

FIG. 1. Structures of cannabinoids tested.

MATERIALS AND METHODS

Inhibitors. Samples of CBN, CBD, CBC, CBCy, Δ^1- and Δ^6-THC were obtained from the National Institute of Mental Health and were 93 per cent pure or better. Stock solutions were periodically assayed by GLC to monitor for decomposition. CBD acid was provided by Prof. R. Mechoulam, Hebrew University, Jerusalem, and was monitored by TLC. Olivetol was obtained from Aldrich Chemical Co.; Naproxen and Demerol were gifts from Syntex Corp. and Sterling–Winthrop respectively. LSD was supplied by Dr. J. Bergen.

The crude marihuana extract used in these experiments was provided by the National Institute of Mental Health and contained the following amounts of cannabinoids: Δ^1-THC (18·7%), Δ^6-THC (1·6%), CBN (6·0%) and CBD (3·9%). The extract is prepared by alcohol extraction of marihuana, followed by thermal decarboxylation (70°) of the acids and hexane partitioning.

Other materials. The 8,11,14-eicosatrienoic acid used was obtained from Applied Science Laboratories, State College, Pa., as was the ^{14}C labeled material which had a specific activity of 55·5 Ci/mole. PGE_1 was a gift of the Upjohn Co., Kalamazoo, Mich. ^3H-PGE_1 was purchased from New England Nuclear Corp., Boston, Mass., and had a specific activity of 110 Ci/mM. All other substances were of reagent grade purity.

Preparation of microsomes. Frozen bull seminal vesicles were obtained from Pel-Freeze Co., Rogers, Ark., and after thawing were homogenized in a Waring blender in 0·25 M sucrose (3 ml/g tissue). The homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant was centrifuged for 1 hr at 100,000 *g*. The microsomal pellet thus obtained was lyophilized and stored at −20°.

Incubation procedure. The procedure was essentially the same as that used previously[1] except that lower concentrations of both the precursor and microsomal powder were used. Five mg of the lyophilized microsomes was preincubated for 5 min at 37° in 1 ml of a solution adjusted to pH 8·1 containing 65 μmoles of EDTA, 5 μmoles of reduced glutathione, 1·0 μmole of epinephrine and 10 μl of ethanol with or without the inhibitor being studied. The reaction was initiated by the addition of 30 μg of ^{14}C-8,11,14-eicosatrienoic acid (5500 dis/min) in 0·1 ml of EDTA and incubated in air for 10 min at 37° with vigorous shaking. The reaction was stopped by adding 1 ml of 1 N H_2SO_4 followed by 18 ml of chloroform–methanol (2:1).

Extraction and analysis. The extraction mixture contained in addition to the solvents 100 μg of ^{3}H-PGE_1 (100,000 dis/min) which acted as a carrier and provided an index for recovery of the ^{14}C-PGE_1 produced by the synthetase. After thorough shaking, the organic phase was removed and washed with 8 ml of 0·025% H_2SO_4, dried over anhydrous Na_2SO_4 and evaporated under nitrogen.

The residues were then dissolved in 0·5 ml of 1 N KOH in methanol and maintained at 37° for 30 min converting the PGE_1 to PGB_1. The mixture was acidified to pH 3·0 with 4 ml of dilute acetic acid and extracted with 5 ml of ethyl acetate, dried over anhydrous Na_2SO_4 and evaporated.

The extracts were chromatographed on Silica gel thin-layer plates in a chloroform–methanol–acetic acid (90:5:5) system and the PGB_1 was located by ultraviolet absorbtion and spraying with phosphomolybdic acid. The appropriate areas were removed from the plates and assayed for ^{14}C/^{3}H ratio by liquid scintillation counting in a toluene–ethanol mixture.

RESULTS

In the previous report of this series, the enzyme source was fresh sheep seminal vesicles which gave a high rate of synthesis. A more readily available source of active tissue was found in frozen bull seminal vesicles (BSV). Tomlinson *et al.*[8] were able to measure the effects of several inhibitors using BSV and we likewise found this to be a satisfactory stystem. Figure 2 shows the relationship beween the amount of enzyme and the rate of synthesis. Our system was also responsive to the effects of inhibitors described in the literature as shown by the examples in Table 1.

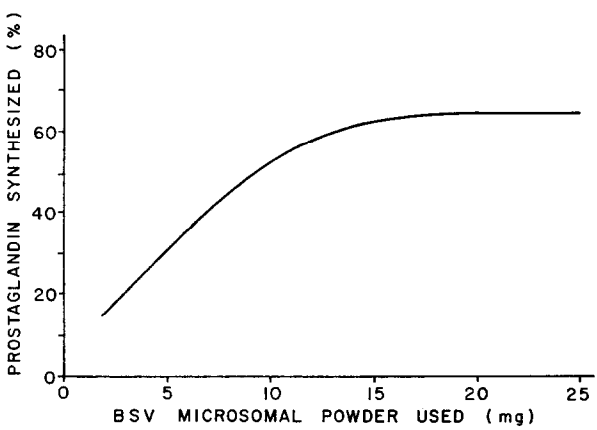

FIG. 2. Effect of enzyme concentration on prostaglandin biosynthesis.

TABLE 1. COMPARISON OF INHIBITORS*

Substance	Concn (μg/ml)	Concn (M)	Inhibition (%)
Δ^1-THC	100	$3{\cdot}2 \times 10^{-4}$	50
Naproxen	30	$1{\cdot}3 \times 10^{-4}$	67
Indomethacin	0·30	$0{\cdot}84 \times 10^{-6}$	58

* The values in this table are the averages of several experiments carried out as described under Methods. Naproxen and indomethacin were tested only at the concentrations shown, hence the ID_{50} was not determined.

We chose to carry out the present experiments with 8,11,14-eicosatrienoic acid the precursor of PGE_1 for two reasons. First, we sought to examine the generality of the inhibition previously observed with Δ^1-THC on PGE_2 synthesis from arachidonic acid. Second, solutions of arachidonic acid are less stable than the trienoic acid.*

A further modification of the previous procedure was in the method of analysis. We had observed occasional partial conversion of the PGE_1 to PGA_1 and PGB_1 during the extraction and analysis. To obtain more consistent results, treatment with alkali was introduced, thereby quantitatively converting the PGE_1 to PGB_1. We also added carrier PGE_1 at the end of the incubation to minimize losses. ^{3}H-PGE_1 was added to measure recoveries.

Table 2 summarizes the results we have obtained with some of the major cannabinoids. There seems to be little correspondence between inhibitory power and psychoactive properties and, although the precision of these experiments is not very high, the trend of the data indicates a reverse relationship. Our findings may be a reflection of the tissue source from which the synthetase is derived. A recent report by Flower and Vane[9] would seem to indicate that, while the qualitative effects of several inhibitors may be observed in different systems, relative potencies may vary by considerable amounts. They showed that, while aspirin was 15-fold more potent than 4-acetamidophenol in dog spleen, they were of approximately equal potency in rabbit brain. If a similar situation exists in our case, then the choice of the "right" system may yield a better correlation. There is also the possibility that inhibition of PG synthesis may be an independent property of THC which is responsible for some of its other actions.

If our data have significance *in vivo*, then some of the cannabinoids such as CBD and CBN should show other pharmacological effects. While these substances have not been studied as thoroughly as Δ^1-THC, there are reports which suggest that this may be the case. Mechoulam and Gaoni[7] have summarized these and, in addition, have reported experiments which indicate that both cannabichromene[10] and cannabigerol[11] possess biological activity. Razdan and Pars[12] have also reported activity for cannabichromene, cannabicyclol and iso-THC. These reports support our findings that several of the cannabinoids inhibit PG synthesis.

We have also attempted to determine whether a portion of the cannabinoid structure is primarily responsible for the inhibition of PG synthesis which we have observed. In structural terms, the cannabinoids can be considered as made up of a terpene

* Dr. John Paulsrud, private communication.

TABLE 2. INHIBITION OF PGE[1] SYNTHESIS BY SOME OF THE NATURAL CANNABINOIDS

Substance	ID_{50}* (μg/ml)	ID_{50}* (M $\times 10^{-4}$)	Correlation	Psychoactivity†
Cannabinol	22	0·70	0·96	−
Cannabidiolic acid	43	1·20	0·99	−
Δ^6-THC	64	2·04	0·72	+
Cannabidiol	69	2·20	0·74	−
Cannabichromene	83	2·64	0·79	−
Δ^1-THC	100	3·18	0·86	+
Crude marihuana extract	173		0·47	+
Cannabicyclol	>100	>3·18		−

* These values represent the average of at least two experiments in which inhibition was measured at 1, 10 and 100 μg/ml of inhibitor in triplicate. The results were evaluated by a computer generated linear plot of PG synthesized vs log concentration of inhibitor. Correlation refers to the degree of fit of the experimental values to the resulting line.

† Based on behavioral effects in the rhesus monkey as described in Ref. 4.

moiety and a substituted resorcinol (Fig. 3) and, in fact, the various laboratory syntheses are based on this principle. When we tested olivetol (3-pentylresorcinol), we found, surprisingly, that it showed a higher potency than most of the cannabinoids thus far examined (Table 3). We have tested *d*-limonene and *p*-cymene as representatives of the terpene portion of the cannabinoids. In a formal sense, *d*-limonene represents part of the CBD molecule except that the endocyclic double bond corresponds to the Δ^6 position; *p*-cymene corresponds to part of CBN. No activity was seen with either even at high levels suggesting that, while the terpene portion may modify activity, the critical portion of the cannabinoid structure is the aromatic moiety.

TABLE 3. INHIBITION OF PGE_1 SYNTHESIS BY COMPOUNDS EITHER STRUCTURALLY OR PHARMACOLOGICALLY RELATED TO THE CANNABINOIDS

Compound	ID_{50} (μg/ml)	ID_{50} (M $\times 10^{-4}$)	Activity
d-Limonene	>100	>7·35	None
p-Cymene	>100	>7·36	None
Olivetol	18	1·0	None
Mescaline	>100	> 4·74	Hallucinogenic
Meperidine	>100	>3·52	Analgesia
LSD	120	3·72	Hallucinogenic

Finally, we examined the possibility that other drugs which act on the nervous system may suppress PG synthesis (Table 3). Meperidine is a potent analgesic which is widely used in medicine. At levels up to 100 μg/ml, no significant inhibition could be seen in our system. Mescaline has long been used as hallucinogen, and it also had no effect on PG synthesis. LSD did show some inhibitory power; however, considering

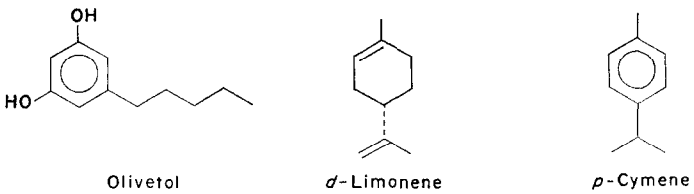

FIG. 3. Structural components of the cannabinoids tested.

its extremely high potency as a psychotomimetic, a greater response would be anticipated if this were its mode of action. It seems then that we are seeing a reasonably specific effect which only certain drugs will exhibit.

In conclusion, the results presented above show that several cannabinoids are capable of suppressing the biosynthesis of PGE_1 under the conditions described. The portion of the molecule responsible for the action seems to be the olivetyl moiety with the terpene group moderating inhibition to different degrees. In the present system, there is poor correlation between inhibitory power and psychoactive properties; however, a more relevant enzyme source may give a quantitatively different picture. We would like to stress that our data may be related to some of the actions of Δ^1-THC and the other cannabinoids which have not been shown to be directly connected with the psychoactive effects of cannabis.

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