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Activation of phospholipase A_2 by cannabinoids

Lack of correlation with CNS effects

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Cannabinoids Δ^1-tetrahydrocannabinol, cannabinol, cannabidiol and cannabigerol have been shown to affect directly the activity of phospholipase A_2 in a cell-free assay. The compounds produced a biphasic activation of the enzyme, with EC_{50} values in the range $6.0–20.0 \times 10^{-6}$ M and IC_{50} values in the range $50.0–150.0 \times 10^{-6}$ M. These results correlated well with the relative potencies reported for the stimulation of prostaglandin release from human synovial cells in vitro, confirming that activation of phospholipase A_2 is the predominant action of cannabinoids on arachidonate metabolism in tissue culture. However, since Δ^1-tetrahydrocannabinol is unique among these compounds in possessing cataleptic activity, it is unlikely that phospholipase A_2 is the major receptor mediating the psychotropic effects of cannabis.

Cannabis; Phospholipase A_2; Catalepsy; Psychotropic effect

1. INTRODUCTION

There is evidence that prostaglandins (PGs) are mediators of the central effects of Δ^1-tetrahydrocannabinol (THC), the psychoactive constituent of *Cannabis sativa* L. For example, the cataleptic effects of THC in mice are abolished by treatment with aspirin, indomethacin, diflunisal and phenylbutazone, agents which inhibit PG synthesis. Catalepsy can be restored by the administration of exogenous PGEs [1]. In addition, the stimulation of arachidonate metabolism by cannabinoids in cell culture systems has been correlated with the psychotropic potency of THC analogues [2]. Burstein and co-workers [3] have proposed that THC interacts with specific sterol binding sites in the plasma membrane, causing activation of phospholipase A_2 and cycloxygenase. Since it is probable that the cannabinoids affect the activity of several enzymes in the arachidonate cascade [4], it was decided to investigate the actions of these compounds on phospholipase A_2 in a cell free system, and to determine whether direct effects on this enzyme were responsible for the observed pharmacological activity of cannabinoids both in vitro and in vivo.

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2. MATERIALS AND METHODS

Δ^1-Tetrahydrocannabinol was obtained from Sigma. Other cannabinoids were purchased from Makor Chemicals, Tel Aviv. Structures are illustrated in fig.1. The ability of cannabis constituents to inhibit the phospholipase A_2 of *Naja naja* venom (Sigma) was determined using a modification of the spectroscopic technique of Wells [5]. Acid release was detected by the formation of the acidic species of bromothymol blue, and enzyme activity determined by measuring the

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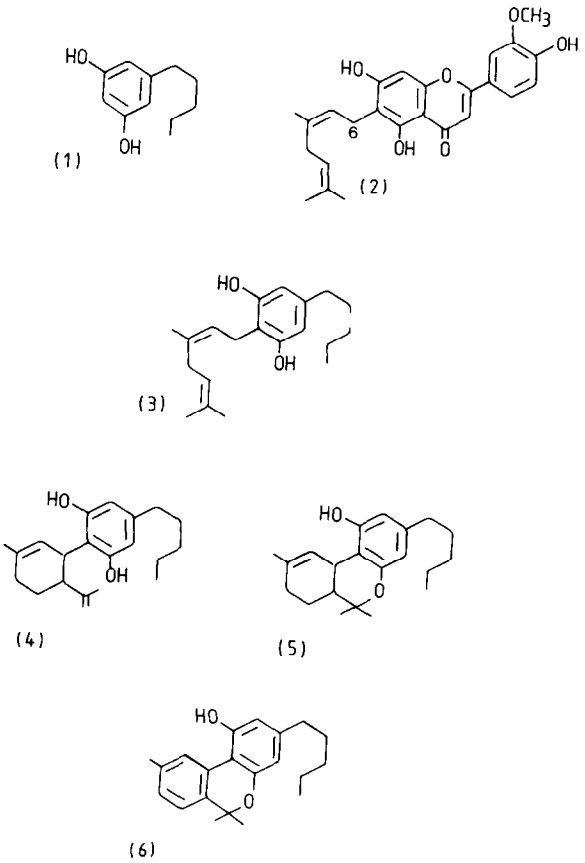

Fig.1. Structures of cannabis constituents. (1) Olivetol, (2) cannaflavin A, (3) cannabigerol, (4) cannabidiol, (5) cannabinol, (6) Δ^1-tetrahydrocannabinol.

rate of increase in absorbance at 445 nm. The assay mixture contained 50 mM bromothymol blue, 5 mM dicapryloyllecithin and 0.05 mM $CaCl_2$ in a volume of 2 ml. The pH was adjusted to 8.0 with NaOH and the reaction initiated by the addition of 1 μg enzyme (equivalent to 0.1 units of activity). At 30°C, the reaction rate was linear over a range of 0.06 absorbance unit (equivalent to the formation of 0.25 μmol acid). Compounds to be tested were added to the assay mixture in 10 μl absolute ethanol prior to the addition of enzyme.

3. RESULTS

The effects of cannabinoids on phospholipase A_2 activity are summarised in table 1 and fig.2. All

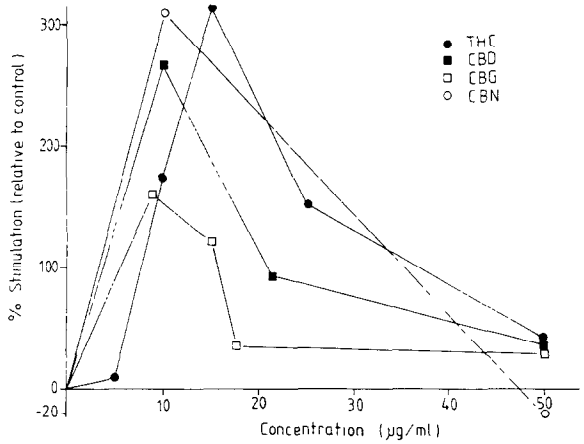

Fig.2. Stimulation of phospholipase A_2 activity by cannabinoids. Details of assay conditions are given in the text. See also table 1. Acid release in the absence of drugs: 0.07 ± 0.006 μmol/min.

Table 1

Biphasic stimulation of phospholipase A_2 activity by cannabis constituents

Compound	Maximum stimulation (%)	EC_{50}		IC_{50}	
		μg/ml	μM	μg/ml	μM
Olivetol	none	–	–	–	–
THC	316	6.0	19.1	48	153
CBD	262	2.0	6.4	42	134
CBN	310	2.0	6.4	41	142
CBG	160	3.0	9.5	17	55
Mepacrine	−90[a]	–	–	6.8	14

[a] Inhibitory activity

Acid release from dicapryloyllecithin due to the action of phospholipase A_2 from *Naja naja* venom was detected using the indicator bromothymol blue. Details of assay conditions are given in the text. Acid release in the absence of drugs: 0.07 ± 0.006 μmol/min. Data are the mean of at least two separate experiments

the compounds tested were potent stimulators of enzyme activity, with concentrations producing 50% maximal stimulation (EC_{50} values) being in the range 5.0–20 × 10^{-6} M. The response was biphasic however, with a decline in activity being observed at higher dose levels. Concentrations at which enzyme activity had returned to 50% max-

Table 2

Correlation of the effects of cannabinoids on phospholipase A_2 with in vitro stimulation of PGE_2 release from human synovial cells, and with cataleptic activity in mice

Compound	Phospholipase A_2		PGE_2 release[a]		Cataleptic activity[b]
	% max.	Concn	% max.	Concn	
THC	316	16	330	30	+ +
CBN	310	12	220	10	0
CBD	262	10	180	10	0
CBG	160	9	inhibition	–	0

[a] Potentiation of TPA-stimulated PGE_2 release from human synovial cells in vitro [6]. Plastic adherent synovial cells were stimulated to release PGE_2 with 10 ng/ml TPA, and after 48 h incubation, the PGE_2 content of supernatant fluids was measured by direct radioimmunoassay

[b] Induction of cataleptic state in mice assessed on an arbitrary scale by the ring test [7]. Compounds were administered orally to male CD1 mice up to maximum tolerated doses, and motor activity assessed by counting the number of times unrestrained animals stray outside a designated area

% max., percentage maximum stimulation above controls; Concn, concentration (μg/ml) of drug at which maximum stimulation was observed

imal (IC_{50} values) were in the range 40–150 × 10^{-6} M. It is noteworthy that THC was the least potent of the four compounds tested on the basis of EC_{50} values, and although THC produced the highest level of stimulation, it was not significantly better than either CBD or CBN.

The results from this cell-free system correlate well with the observations of Barrett and co-workers [6] who studied the effects of cannabinoids on human synovial cells. They demonstrated a biphasic stimulation of PGE_2 release by THC, CBD and CBN, whilst CBG was inhibitory. In contrast, we have confirmed that THC is unique among the cannabinoids in possessing cataleptic activity in the ring test [7]. These comparisons are summarised in table 2.

4. DISCUSSION

Using an in vitro assay, we have observed a direct effect of cannabinoids on phospholipase A_2 from *Naja naja* venom (fig.2). The use of a short chain lecithin as substrate allowed activity to be demonstrated without the addition of detergents which may affect the action of certain drugs. It is significant that mepacrine possesses inhibitory activity in this system (table 1), whereas it is generally ineffective in hydrolysis procedures using high molecular mass lecithins which require solubilisation by detergents [8]. The relative potencies of the four cannabinoids, THC, CBD, CBN and CBG (table 1) correlated well with data previously reported for at least one in vitro system (human synovial cells) and it may be concluded that stimulation of phospholipase A_2 is the most significant step in initiation of prostaglandin release. Our results resolve the mechanism of action of these compounds to three possible sites. Firstly, they may affect calcium activation of the enzyme. Since the cannabinoids have been shown not to affect the activity of another calcium-dependent enzyme, protein kinase C (not shown), it is unlikely that these compounds exert a unique action on the calcium dependence of phospholipase A_2. Second-

ly, the amphipathic nature of these molecules might allow their intercalation into the substrate micelle (or protein-phospholipid environment of the cell membrane) creating greater availability of substrate, followed by enzyme stimulation, and at higher concentrations a decrease in effective substrate concentrations and inhibition. This is also unlikely to be the major mechanism of action since stereospecific structural requirements have been reported for activity in cell culture (Burstein, S., personal communication). We therefore suggest that the cannabinoids interact with a hydrophobic regulating site on phospholipase A_2, and that this site is present on both soluble and membrane forms of the enzyme.

A striking feature of these results is the similarity of the actions of cannabinoids on prostaglandin metabolism in vitro, contrasted with the unique central effects of THC. Inhibitor studies in vivo have produced convincing evidence that PGs are involved in mediating the cataleptic response to THC in mice [1]. However, PGs play a role in regulating the expression of various receptors in the brain [9] and recent studies have demonstrated a major role of catecholamine pathways in mediating behavioural and antinociceptive effects of cannabis [10,11]. Thus, whilst it is possible that the greater stimulatory effect of THC on phospholipase A_2 in vitro is sufficient to explain the dramatic difference in pharmacological profile, it is more likely that the THC receptor is not located in the arachidonate cascade itself, but in a pathway which is sensitive to changes in PG levels.

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