THE ROLE OF PROSTAGLANDINS IN THE ACTIONS OF THE CANNABINOIDS

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I. INTRODUCTION

The molecular events surrounding the interactions of cannabinoids with various tissues remain ill-defined despite more than a decade of intense activity by many investigators. A number of hypotheses have arisen during this period (1), however, many of the observed pharmacological effects of the cannabinoids still are lacking a molecular model. Several of the proposed sites of action involve membrane-bound enzymes and it has been suggested that the cannabinoids exert their effects by changing the nature of the lipid environment surrounding these enyzmes. Data from our laboratory has given rise to the possibility of an additional membrane site for cannabinoid action (2).

Our findings have pointed to the activation of phospholipases as a likely primary site for the initiation of events (3) that could result in the production of a number of mediators with a spectrum of activities (4). This involves the release of phospholipid-bound arachidonic acid which then becomes available for bioconversion to a variety of potent regulatory substances, such as, prostaglandins (PGs), prostacyclin, leukotrienes, etc.; this process has acquired the name, the arachidonic acid cascade. Phospholipases are thought to be involved as physiological regulators in these

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bioconversions, in particular, phospholipase A_2 has been implicated in the synthesis of PGs in several systems. An alternate mechanism has recently been suggested known as the "PI" hypothesis (4). In this type of regulation, phospholipase C acts on phosphatidylinositol to generate a diacylglyceride, which is then enzymically converted to the monoglyceride and free arachidonic acid. The two mechanisms seem to be either tissue specific or agonist specific and could account for the physiological and pharmacological specificity observed with the arachidonic acid cascade.

Observations from several laboratories using *invivo* or other complex systems suggested that the cannabinoids were able to stimulate the production of PGs. An interesting set of experiments has been reported by Fairbairn and Pickens using THC induced catalepsy in the mouse as an endpoint (5). They found that the cataleptic effect of the drug could be blocked by pretreating the mice with aspirin or other inhibitors of PG synthesis. If the animals were then injected with PGE_2, the catalepsy could be restored. In another study they reported that mice raised on a diet deficient in essential fatty acids did not respond to the delta-9-tetrahydrocannabinol (THC), however, the administration of arachidonic acid rapidly restored the cataleptic response (6).

We have demonstrated an analogous effect using THC induced hypotension in dogs as the test system (7). Table I shows that pretreatment of pentobarbital anesthetized dogs with aspirin greatly reduces the hypotensive action of THC. A likely explanation for these findings is that THC is stimulating the synthesis of an arachidonate-derived vasodilator, such as prostacyclin, either in the lungs or the peripheral vascular bed. Aspirin is known to effectively block this process (8), thereby preventing THC released arachidonate from being converted to prostacyclin.

A role for prostaglandins in the cannabinoid effects on the pituitary-testicular axis in mice has been suggested by Dalterio et al (9). Under specific conditions, they found that both PGE and PGF synthesis in the pituitary and testis were stimulated by THC, CBD, and CBN. The data were obtained by immunoassays for the PGs from incubation of the tissues following *in vivo* drug treatment.

Direct measurement in the rat brain of "PGE_2-like" material following THC using a bioassay procedure was reported by Coupar and Taylor (10). They found no changes in most brain regions except the hypothalamus where there was a small decrease. Considerable methodological difficulties have been reported in measuring brain levels of PGs (11), so that these results should be viewed with some caution. A number of earlier reports on cannabinoid-PG effects have been reviewed already (1) and will not be discussed here.

TABLE I. Inhibition of THC-induced Hypotension by Aspirin

Time[a]	Decrease in mean arterial blood pressure (+/- S.D.) (mm Hg)		%I[d]	P
	THC[b]	Aspirin-THC[c]		
5	38.5 (23)	7.25 (4.6)	81	< 0.0005
10	62.3 (22)	17.5 (12)	72	< 0.0005
15	71.8 (23)	27.8 (17)	61	< 0.005
20	75.0 (18)	38.5 (15)	49	< 0.005
30	67.0 (13)	42.5 (19)	37	< 0.05
40	60.5 (14)	39.3 (19)	35	> 0.05

[a]Post THC injection times.
[b]Intravenous dose of 0.45 mg/kg in 0.45 ml EtOH; N = 4.
[c]Intravenous dose of 50 mg/kg in 5 ml H_2O at pH 7.3, 30 min prior to THC; N = 4.
[d]Percent inhibition of the hypotensive effect by aspirin.
Ref.: 20.

For some time now we have been studying PG-cannabinoid interactions in an attempt to describe the biochemical basis of the effect. Our earlier studies using cell culture systems suggested that THC somehow activated a phospholipase, releasing arachidonic acid which then was converted to PGs (3). These experiments were done primarily with Hela cells although some data was obtained using suspensions of mouse Leydig cells. The data demonstrated that THC was effective in releasing ^{14}C-arachidonate from labelled phospholipid pools, and that this could be blocked by the use of lipase inhibitors such as mepacrine (12). The precise identity of the phospholipid(s) involved is not known nor has its cellular location been determined. These points are of obvious mechanistic importance and will be the subject of future studies.

More recently, we have utilized a different cell line for this investigation, namely, the WI-38 human lung fibroblast (13). This choice was governed by several considerations. Preliminary experiments had shown that these cells were highly sensitive to THC at 3.2 mcM giving an 80% increase of arachidonate release over control values (3). Moreover, these cells were of human origin from an organ where cannabinoids are known to concentrate making them relevant to <u>in vivo</u> effects. Finally, they are a well-established easily maintained system about which a great deal is known, especially the effects of THC on c-AMP metabolism (14).

II. DISCUSSION

The dose-response relationship for PGE stimulation by THC in the WI-38 fibroblast is shown in Figure 1 (7). It can be seen that, even at the rather modest dose of 1.6 mcM, a two-fold elevation of PGE levels over base line was observed. The exact shape of the curve represents the average of several experiments. In this same report we also showed that the stimulation of PGE levels could be blocked by either aspirin or mepacrine pretreatment. Both of these drugs are well known inhibitors of PG synthesis, the former acting on cyclooxygenase (8) and the latter on phospholipase (12,15).

Our current experiments have centered around the effect of cannabinoid structural features on the release of arachidonate and the subsequent elevation of PG levels (16). We have con-

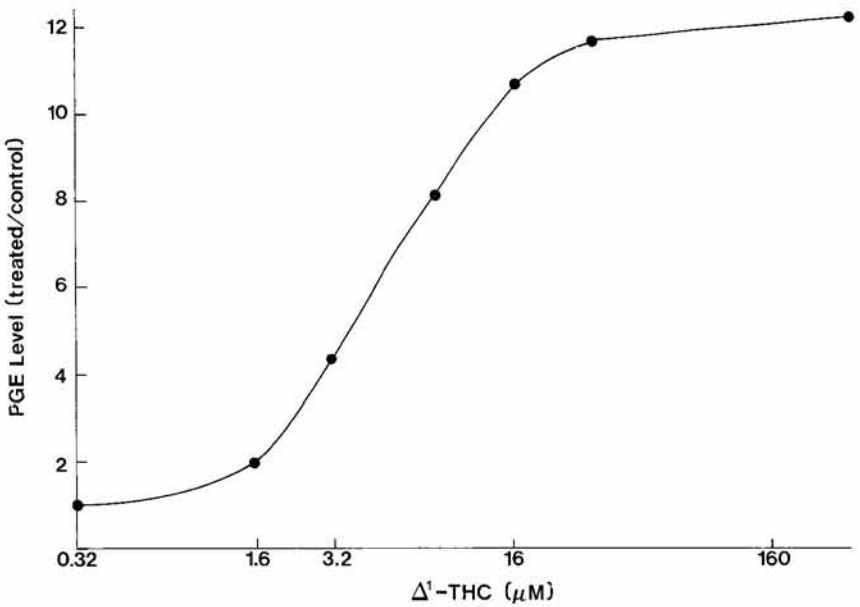

FIGURE 1. Effect of increasing concentrations of THC on PGE_2 levels in monolayer cultures of WI-38 human lung fibroblasts. The cells were grown and treated as described in Ref. 7. Each data point is the mean of six determinations. The stimulated cells were significantly different from controls at the $P < 0.0005$ level using Student's t-test ($N = 6$) at each dose level tested.

tinued to use the WI-38 cells as before and have measured the drug effects by both the radiolabelled precursor method and immunoassay measurement of the release of endogenous PGE. The effect of the cannabinoids on cell viability was also studied since visual observation had suggested that high doses caused such changes. Figure 2 gives several examples of the relationship of drug dose to DNA content, which was our criterion for cell viability. The three primary cannabinoids THC, CBN, and CBD, caused marked changes in viability starting at around 8mcM; by contrast, the 7-hydroxy metabolite of THC had no measurable effect on the cells even at 32 mcM.

The effect of metabolic changes in THC structure on PGE levels is rather interesting and is summarized in Figure 3. Unmetabolized THC is the most potent while the 7-carboxy

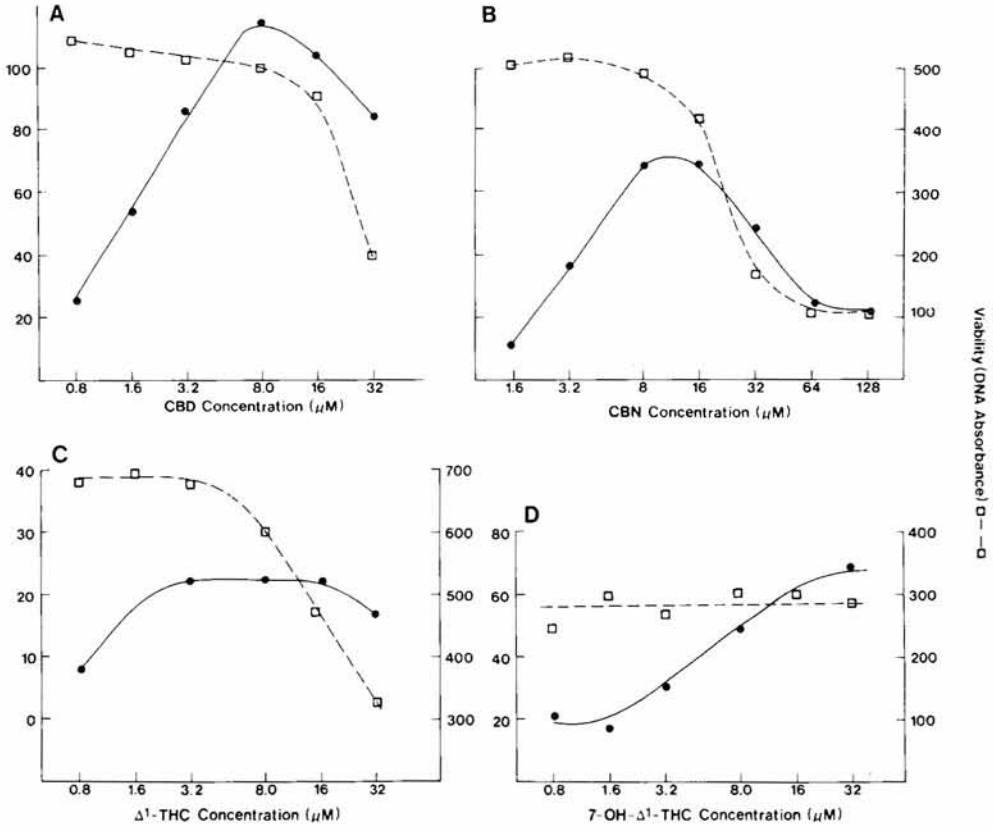

FIGURE 2. Cannabinoid effects of fibroblast viability compared with PGE production.

derivative, a metabolic end product, is essentially inactive. The other products give a spectrum of intermediary activities. This parallels in an approximate fashion some of the in vivo activities of the substances (17-19). These results suggest that the prostaglandin effect is related to cannabinoid actions in vivo, or that they are each mediated by similar molecular events.

If the release of arachidonic acid by the cannabinoids is responsible for the stimulation of PG synthesis, there should be a good correlation between their individual potencies in both effects. Figure 4A shows that this is indeed the case for every substance tested except cannabicyclol (CBCy). The data were obtained by measuring released ^{14}C-arachidonic acid and released ^{14}C-PGE$_2$ following separation by thin layer chromatography. The nonconformity exhibited by CBCy may be related to the fact that it is the only compound of the group which cannot assume a planar conformation.

Figure 4Bshows similar results on a smaller series of cannabinoids which probably accounts for a somewhat less perfect correlation (R = 0.849). In this experiment, ^{14}C-arachidonate is compared with PGE measured by immunoassay. The latter would arise from the endogenous pool(s) of arachidonate

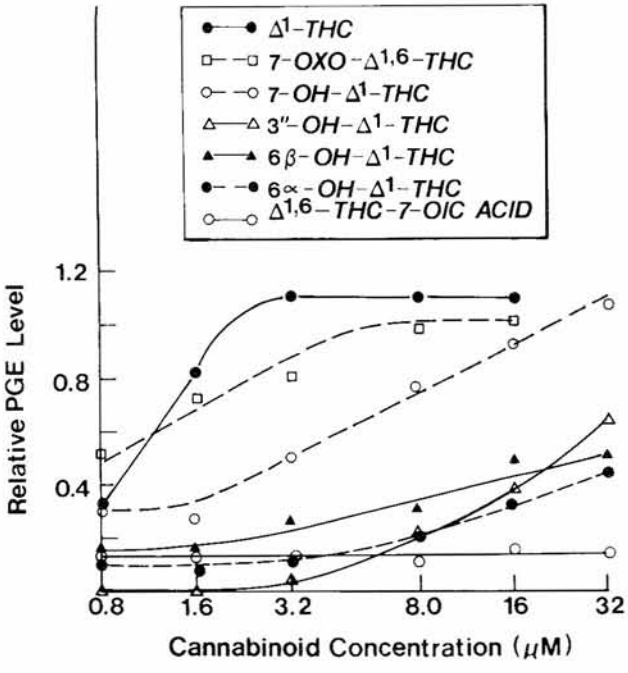

FIGURE 3. The effect of metabolism on cannabinoid stimulation of PGE synthesis in fibroblasts.

indicating that the radiolabelled precursor method accurately monitored the actual cell response.

The order of activites of the primary cannabinoids we observed is unrelated to their known *in vivo* actions. At this time we have no explanation for this, however, it may well be related to distribution and metabolism considerations. It is worth noting that White and Tansik have also observed cannabinoid mediated stimulation of arachidonate and PG release in ^{14}C-labelled platelets (16). Interestingly, they also found that CBD was about 1.5 times more potent than THC.

The question of what role, if any, the prostaglandins may have as mediators of cannabinoid action(s) can now be approached with relative certainty in view of the data discussed above. These reports from other laboratories as well our own allow several conclusions to be made. First, there is strong evidence that central nervous system effects, such as, catalepsy and pituitary secretion involve PGs as mediators. Secondly, effects on the cardiovascular system, in particular arterial blood pressure, can be explained by cannabinoid induced stimulation of vasodilatory products of the arachidonic acid cascade, such as prostacyclin. Thirdly, drugs, such as aspirin and other nonsteroidal anti-inflammatory agents, should be clinically effective anti-cannabinoids. Mepacrine may also exhibit this property due to its phospholipase inhibition. Finally, *in vitro* systems such as cells in culture or other systems which contain membrane assemblies seem to provide relevant models for studying cannabinoid action at the molecular level.

Of the many actions of the cannabinoids, the mood altering effect of the tetrahydrocannabinols presents the greatest interest in terms of cannabis use and abuse. At this point it is not possible to say whether prostaglandins do, in fact, play a role in this behavioral response in which man is the only truly valid model. It would be of interest to see whether some of the findings discussed above can be brought to bear on this problem. For example, does aspirin usage have any effect on the nature or intensity of the marijuana "high"?

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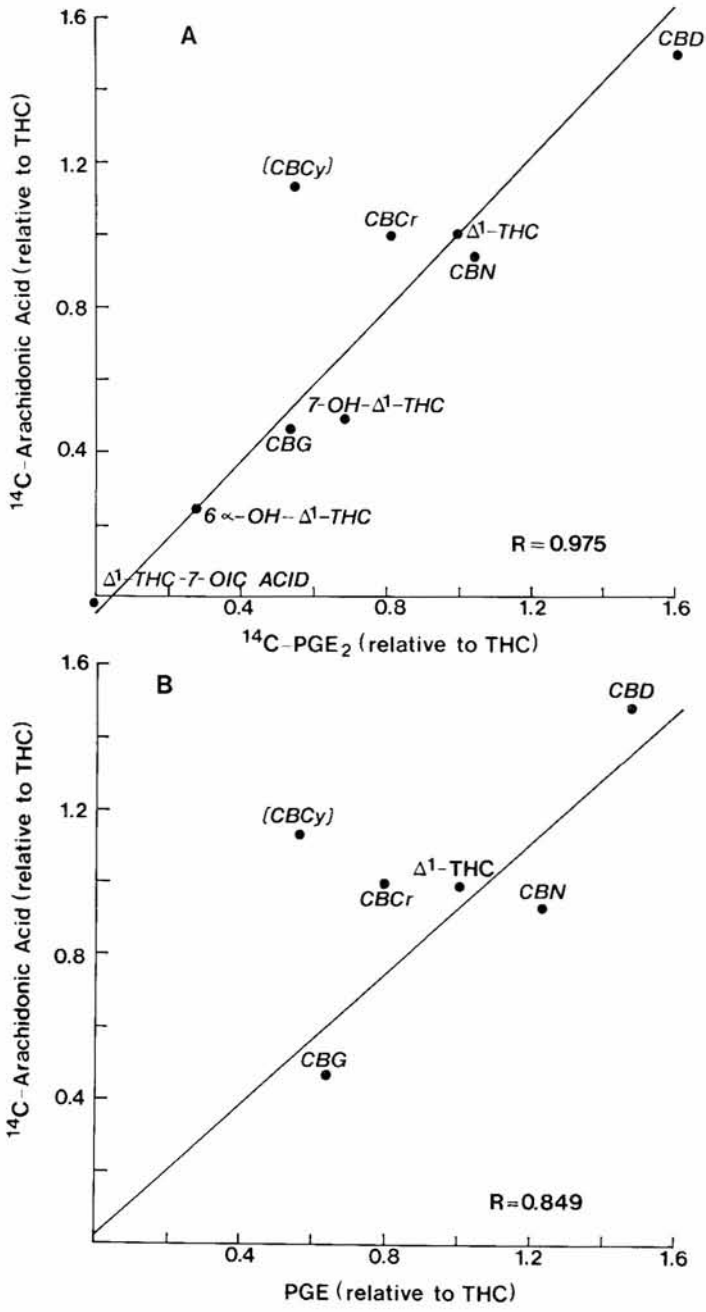

FIGURES 4A & 4B. Comparison of arachidonic acid release and PGE synthesis in fibroblasts.

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