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PROSTAGLANDINS AND CANNABIS—XIII

CANNABINOID-INDUCED ELEVATION OF LIPOXYGENASE PRODUCTS IN MOUSE PERITONEAL MACROPHAGES

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Abstract—The phospholipases controlling the release of arachidonic acid in mouse peritoneal macrophages have been shown to be stimulated by the natural psychoactive cannabinoids. A close correlation was observed between the potencies of these substances in elevating arachidonate levels *in vitro* and the reported activities in a behavioral assay in monkeys and in producing a "high" in humans. The order of activity with the macrophages was Δ^1-tetrahydrocannabinol (Δ^1-THC) > 7-OH-Δ^1-THC > 6α-OH-Δ^1-THC > 6β-Δ^1-THC >> Δ^6-THC-7-oic acid. It is suggested that this effect, which has now been shown in several diverse cell types, may serve as a model for studying the mechanism of action of THC.

Earlier studies reported by us demonstrated that the cannabinoids are effective agents in stimulating the release of arachidonic acid from fibroblasts [1, 2]. We also reported that a direct result of this increase in free arachidonate is a dramatic rise in prostaglandin (PG) synthesis by these cells. A comparison of released arachidonic acid and PGE_2 synthesis in this model suggested that the cannabinoids were stimulating the lipases regulating the arachidonic acid cascade rather than cyclooxygenase or subsequent enzymes in the pathway [2].

A second pathway for arachidonate metabolism is catalyzed by lipoxygenase enzymes and has been studied extensively in recent years [3, 4]. This route leads to a variety of potent mediators of cell function such as the leukotrienes and other hydroxyeicosatrienoic acids (HETEs) whose tissue levels seem to depend on the activities of the phospholipases which control free arachidonic acid levels as with the prostaglandins. A model which has been used in a number of laboratories for studying the lipoxygenase pathway is the peritoneal macrophage [3, 4]. The uptake of labeled arachidonic acid into various phospholipids and its conversion to both cyclooxygenase and lipoxygenase products have been reported in the literature [5, 6]. Coincidently, this same cell type has been reported to be sensitive to the actions of cannabinoids as measured by morphological changes [7] and by inhibition of an acyltransferase in the closely related splenic lymphocyte [8].

Based on the above considerations, we anticipated that the cannabinoids would cause increased lipoxygenase-mediated synthesis in macrophages by virtue of their ability to increase the availability of free arachidonate. We were also interested to see whether a structure–activity relationship similar to that found in the fibroblast would be observed; in that model we showed good correlation between PG synthesis and literature values for behavioral responses. The present report describes our findings on the effects of Δ^1-tetrahydrocannabinol (THC) and several of its metabolites on the release of arachidonic acid from unstimulated mouse peritoneal macrophages.

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MATERIALS AND METHODS

Chemicals. The cannabinoids were obtained from the National Institute on Drug Abuse and their purity was monitored by reversed phase thin-layer chromatography. Whatman KP-18 plates were used and the solvent system was 50% ethanol, 37.5% methanol, 12.5% water (pH 4.0). Each cannabinoid gave a single spot where visualized with iodine vapor. Bovine serum albumin, Fraction V, was purchased from the Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]Arachidonic acid (sp. act. 52.7 mCi/mmole) was obtained from the New England Nuclear Corp. (Boston, MA).

Preparation of macrophages. The cells were obtained by peritoneal lavage with Earle's minimum essential medium (MEM) from 50-day-old male mice (Charles River CD-1). Ten mice were used for each experiment yielding approximately 50 × 10^6 cells in 50 ml MEM to which penicillin-streptomycin (100 units/ml-100 μg/ml) was added. The cell suspension was divided, added to 24 "miniwells" and allowed to attach overnight in an incubator at 37° in an atmosphere of 95% O_2: 5% CO_2.

Labeling with [^{14}C]*arachidonate.* The [1-^{14}C]-arachidonic acid (40,000–60,000 dpm) in 0.5 ml MEM (pH 7.6) was added to each well after removal of the old medium. The uptake was allowed to proceed for 1 hr at 37° during which time 50–70% of the radiolabel was incorporated into phospholipids by the macrophages. The media were then removed, the cells were rinsed with 0.5 ml MEM, and 0.5 ml MEM containing 0.1% albumin was added to each well.

Table 1. Stimulation of arachidonate release by Δ^1-THC

	Dose* (μM)	Carbon-14 (%) ± S.D.†
Control‡		9.23 ± 0.31
Δ^1-THC	0.8	8.12 ± 0.33
Δ^1-THC	1.6	7.38 ± 0.37
Δ^1-THC	3.2	9.67 ± 1.34
Δ^1-THC	8.0	15.1 ± 0.89
Δ^1-THC	16	17.3 ± 1.02
Δ^1-THC	32	18.2 ± 0.42

* Final concentration in the culture media.
† Values are percentages of incorporated radioactivity released into the media. N = 4.
‡ The control consisted of 10 μl of 95% ethanol.

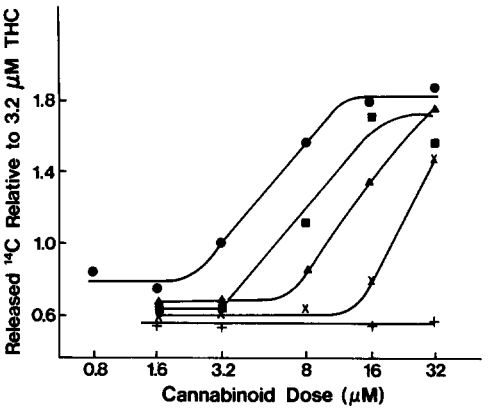

Fig. 1. Release of total radioactivity from [^{14}C]arachidonate-labeled macrophages treated with THC and its metabolites. Values are relative to that obtained with 3.2 μM THC. Key: (●) Δ^1-THC; (■) 7-OH-Δ^1-THC; (▲) 6α-OH-Δ^1-THC; (×) 6β-OH-Δ^1-THC; and (+) Δ^6-THC-7-oic acid.

Release studies. The cannabinoids were added in 10 μl of 95% ethanol to each well and allowed to act for 1 hr at 37°. The control consisted of 10 μl of 95% ethanol. Duplicate aliquots (0.1 ml) of media were then removed and assayed for radioactivity by liquid scintillation counting; quenching was corrected for by the channels ratio method.

RESULTS

Treatment of macrophages labeled by exposure to [^{14}C]arachidonic acid with Δ^1-THC resulted in a dose-related release of radoactivity (Table 1). The effect began at a drug concentration of 3.2 μM and reached a plateau at around 16 μM. Chromatographic analysis (Table 2) of this released radioactivity in the media showed that the major products were hydroxylated metabolites of arachidonic acid (HETEs). Analysis of media extracts from untreated cells showed greatly reduced levels of radioactivity in all chromatographic zones including 6 keto-$PGF_{1\alpha}$, PGE_2, HETE and arachidonic acid.

A comparison of the potencies of Δ^1-THC and four metabolites is shown in Fig. 1. Total released radioactivity was measured over a dose range of 1.6 to 32 μM, and the results are expressed relative to the value shown by THC at 3.2 μM. The order of activities was found to be Δ^1-THC > 7-OH-Δ^1-THC >6α-OH-Δ^1-THC > 6β-OH-Δ^1-THC >> Δ^6-THC-7-oic acid; this last cannabinoid showed no activity above baseline at all doses tested. All of the active compounds exhibited parallel dose–response curves and gave a maximal stimulation about three times above baseline.

DISCUSSION

The findings presented above suggest, as we had expected, that the cannabinoids are potent stimulators of lipoxygenase product synthesis. This is a consequence of the elevation of the levels of free arachidonic acid which is the precursor for both the cyclooxygenase and lipoxygenase pathways [3, 4]. As in our previous model systems, we feel the best explanation for this effect is an increase in the activity of the lipase responsible for releasing free arachidonate in the mouse peritoneal macrophage.

Table 2. Product composition in the media from [^{14}C]arachidonate-labeled macrophages

	^{14}C-Content* (dpm)	
TLC zone	Control†	32 μM THC-treated
1 (Origin	3	201
2	9	140
3 (PGE_2 + 6-keto-$PGF_{1\alpha}$)	43	144
4	64	801
5	171	1053
6 (HETEs)	181	1097
7 (Arachidonic acid)	12	420
Total dpm	483	3856

* See Ref. 2 for extraction and TLC conditions.
† 95% Ethanol.

Table 3. Comparison of arachidonate release with behavioral activity of Δ^1-THC and metabolites

	Subjective "high" in humans			Monkey assay (mg/kg)	ED_{50} in Macrophages (μM)
Δ^1-THC	18.77*	3.5†	100‡	0.1§	4.5
7-OH-Δ^1-THC	16.6	7.5	120	0.5–0.9	8.0
6β-OH-Δ^1-THC	48.64		20	2.0	2.5
6α-OH-Δ^1-THC	>186		25	2.0	13
Δ^6-THC-7-oic acid	>304			>5.0	>32

* Ref. 14. Dose (μg/kg) needed to obtain a "high".
† Ref. 15. Intensity of "high" at equivalent doses.
‡ Ref. 16. Relative potencies using arbitrary scale.
§ Refs. 17–20. The dose needed to produce equivalent responses.
‖ See also Fig. 1. The dose needed to cause 50% maximal release of arachidonate.

Such lipases are generally accepted to be the physiological control points for the synthesis of prostaglandins, thromboxanes and leukotrienes [9]. The other enzymes in these biosynthetic pathways, while sensitive to drug-induced inhibition, have not yet been shown to be subject to either physiological or pharmacological stimuli. The exact molecular nature of this cannabinoid-mediated stimulation is still a matter for speculation. Due to the structural similarities between THC and cholesterol, we have suggested previously [2, 10] that the THC effects on arachidonate release are analogous to those shown by cholesterol in platelets [11].

Raz and Goldman [7] reported some time ago on extensive morphological changes when mouse peritoneal macrophages were treated with THC. These changes were apparently due in large part to membrane perturbations; this could be readily explained by increased phospholipid hydrolysis consistent with our present findings on lipoxygenase product synthesis. A somewhat different conclusion was reached by Mellors [8] using a similar cell type, namely, mouse splenic lymphocytes. He found that acyltransferases in these cells could be inhibited by THC, an action which could also result in elevated levels of free fatty acids. Several considerations make this a less likely explanation, in our opinion, than a stimulation of phospholipases. First, the data were obtained using isolated acyltransferases from a broken cell preparation. It is possible that THC may react preferably with other enzymes in intact cells. Second, the substrate for their study was oleate, not arachidonate, and it is also possible that there are acyltransferases with high substrate specificity [12]. Finally, we have tested the effect of THC on arachidonate uptake in the human lung fibroblasts and found no significant inhibition [13]. Moreover, mepacrine, a specific phospholipase inhibitor, prevented the release of arachidonate by THC [13].

The structure–activity relationship shown in Fig. 1 for the macrophages is very similar to that which we recently reported for cannabinoid-induced stimulation of PGE synthesis in lung fibroblasts [2]. Thus, two quite different cell types exhibit a similar range of sensitivities to THC and its metabolites. Of greater interest is the remarkable correlation shown by these *in vitro* model systems to the available data on the behavioral effects of these cannabinoids (Table 3).

Not surprisingly, the best correlation is with the data reported for the behavioral response in the rhesus monkey [17–20]. The only major difference is in the potencies of the 6α- and 6β-hydroxy metabolites; in the monkey they were equipotent while in the macrophages the 6α showed about twice the activity of the 6β. In both instances, the acid was inactive while THC itself was clearly the most potent substance. The 7-hydroxy metabolite was somewhat less active than THC in our *in vitro* model in agreement with the monkey assay; however, in all three human studies [14–16] this metabolite was more potent as measured on an arbitrary scale of self-reported "high". In agreement with our observations, the acid was inactive and the 6-hydroxy metabolites were intermediate. Despite the differences, the overall picture is one of agreement between the *in vitro* and *in vivo* findings.

There seems little doubt that the prostaglandins have a role in the functions of the central nervous system [21]. However, the nature of the role is, at this time, not well understood. Thus it is not possible, at present, to suggest a specific relationship between our *in vitro* observations and the psychotropic properties of the cannabinoids. One rather important conclusion can be reached with some degree of certainty, namely, that the underlying molecular events responsible for both the *in vitro* and *in vivo* effects must be either identical or at least very similar. As we have suggested above, this may be a cholesterol-like interaction with specific lipids in the membrane bilayer. Physico-chemical evidence for such interactions has been suggested earlier by electron spin [22] and nuclear magnetic resonance studies [23] and has been confirmed recently by microcalorimetry [24]. The cannabinoid effects on arachidonate metabolism appear to be a useful model for the elucidation of the mechanism of action of these drugs and for integrating their physical, biochemical and pharmacological activities.

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