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Effects of Δ^9-Tetrahydrocannabinol on Prostaglandin Formation in Brain

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SUMMARY

In order to investigate the effects of cannabinoids on prostaglandin (PG) formation in the mammalian central nervous system, slices were prepared from different regions of guinea pig brain and incubated with (−)-*trans*-Δ^9-tetrahydrocannabinol (THC) with or without acetylcholine (ACh). The amounts of prostaglandins E (PGE) and (F (PGF) released into the medium were measured by radioimmunoassay. Incubation of cerebral cortex slices for 1 hr with concentrations of THC over the range of 0.8–16 μM significantly inhibited the formation of both PGE and PGF by up to 50% of control levels. Incubation of cortical slices with Δ^8-THC, a psychoactive THC congener, resulted in similar dose-dependent inhibitions in both PGE and PGF levels. Congeners of THC devoid of psychoactivity, namely, cannabidiol and the inactive stereoisomer of THC, were less potent inhibitors of PGE synthesis. In marked contrast to the inhibitory effect of the psychoactive cannabinoids, PGF levels were elevated in the presence of the nonpsychoactive congeners. The effects of THC on PG formation were compared in different brain regions. In striatal slices, 0.8 μM THC significantly stimulated PG formation but did not affect PG levels in the cerebellum. In order to gain insight into the interaction between THC and endogenous neurotransmitters, we compared the effect of THC on the ACh-induced increase in PG formation in the cerebral cortex and the cerebellar cortex. In the cerebral cortex, 0.8 μM THC abolished the ACh-induced increase in PGE levels and inhibited the rise in PGF by 70%. In contrast, THC did not significantly affect the ACh-induced rise in PG levels in the cerebellum. The results indicate that cannabinoids alter both the basal and ACh-enhanced formation of PGE and PGF in the brain and that these effects are structurally and regionally specific.

Although there is much information on the behavioral and cellular effects of the prototypic cannabinoid, THC, its mechanism of action remains elusive. The action of cannabinoids on PG synthesis has been the subject of numerous studies in non-neural model systems. In ram seminal vesicles, concentrations of THC as low as 1 μM inhibited the conversion of exogenous AA to PGs (1–3). Several other studies have shown that THC inhibits PG formation in preparations derived from rabbit kidney (4), rat ovary (5), and mouse testis (6). Inhibition of PG formation by THC has also been observed in synaptosomes (7, 8) and in platelets (9). Conversely, THC increased AA and PG levels in HeLa, mouse Leydig, and human lung fibroblasts in culture (10). In lung fibroblasts, the cannabinoid-induced release of AA exhibited structural specificity, insofar as several metabolites of THC which are devoid of psychoactivity were less potent than THC in releasing AA (11). Based on the above work, it was proposed that lung fibroblasts in culture could be a useful model in studying the mechanism of THC action, although in this system, the nonpsychoactive cannabinoids, cannabidiol and cannabinol, were as effective as the psychoactive congeners, THC and 11-OH-THC, in increasing eicosanoid levels. Thus, the relationship between the effects of cannabinoids on PG formation in non-neuronal cell cultures and their behavioral effects is unclear.

There is, however, strong indirect evidence supporting the view that eicosanoids are involved in mediating some of the central actions of THC. NSAIDs abolished THC-induced catalepsy (12) and attenuated the analgesic, hypothermic, and antiepileptic effects of THC in mice (13). Eicosanoids may also be involved in mediating the hypotensive effect of THC, as pretreatment with aspirin blocks this response in dogs (14). Although these results implicate an enhancement of PG formation in the biochemical pharmacology of cannabinoids, relatively little is known regarding the effects of cannabinoids on PG synthesis in preparations derived from the mammalian brain. In order to determine whether cannabinoids alter PG formation in the brain, we measured the effects of THC and several of its congeners on the formation of PGE and PGF in guinea pig brain slices incubated *in vitro*. Furthermore, in order to gain insight into the interaction between THC and endoge-

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ABBREVIATIONS: THC, (−)-*trans*-Δ^9-tetrahydrocannabinol; AA, arachidonic acid; ACh, acetylcholine; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; KHBS, Krebs-Henseleit bicarbonate saline; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; PGE, prostaglandin E; PGF, prostaglandin F; ANOVA, analysis of variance.

nous neurotransmitters, we studied the effect of THC on the ACh-induced stimulation of PG formation in the cerebral and cerebellar cortices.

Experimental Procedures

Materials. Specific antisera to PGE and PGF (with little or no cross-reactivity for other arachidonate metabolites) were obtained from Miles Immunochemicals (Naperville, IL). Authentic PGE_2 and $PGF_{2\alpha}$ standards were purchased from The Upjohn Company (Kalamazoo, MI), and tritiated PGE_2 and $PGF_{2\alpha}$ were purchased from New England Nuclear (Boston, MA). Acetylcholine chloride, atropine sulfate, BSA (fatty acid free), dextran, and charcoal were obtained from Sigma Chemical Co. (St. Louis, MO). The cannabinoids used in this study, i.e., THC, Δ^8-THC, (+)-THC (the inactive stereoisomer of THC), and cannabidiol, were obtained through the National Institute on Drug Abuse (Rockville, MD). The drugs were stored in ethanol at −20° under N_2. Stock solutions were prepared immediately before use by evaporating the ethanol and resuspending the drugs in DMSO.

Preparation of brain slices. Male guinea pigs (300–450 g) were stunned and then decapitated. The brains were rapidly removed and rinsed in cold KHBS at pH 7.4. Cerebral cortex slices, 0.5 mm thick (weighing on average around 40 mg), were prepared with a Stadie-Riggs tissue slicer. Cortical slices were taken from the surface of each hemisphere and divided into halves or quarters. Similarly, a single slice was taken from the surface of the cerebellar cortex and divided into two sections. Slices from the hippocampus and corpus striatum were obtained from each hemisphere after removing the overlying cortical matter, which clearly exposed these regions. Slices were placed in siliconized flasks with 3 ml of oxygenated KHBS, containing 10 mM glucose and 1% BSA, and incubated in a shaking water bath for 30 min at 37° in an atmosphere of 5% CO_2 in O_2. After removing the medium, the slices were washed two additional times by incubation for 15 min in 1% BSA in KHBS.

Incubation with drugs. Three ml of KHBS were added to the flasks with or without cannabinoids, which were added in 7.5 μl of DMSO, and incubated for 1 hr at 37°. Control samples were incubated in the presence of vehicle alone, which did not affect the basal PG levels. The cannabinoids remained in solution at concentrations up to 80 μM in the presence of 0.25% DMSO. For the experiments measuring the effects of cannabinoids on ACh-induced enhancement of PG release, slices were preincubated with 0.8 μM THC for 5 min, followed by addition of 5 mM ACh (in the presence of 0.1 mM eserine), and further incubated for 30 min. Following the incubation with drugs, the vessels were placed on ice and the medium was removed. In some of the experiments, 0.5-mm-thick cortical slices were obtained from two guinea pigs and cross-chopped into 0.35 × 0.35 mm sections using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY). This method of tissue preparation allowed for preincubation of slices in a single vessel as described above, followed by their suspension in 40 ml of KHBS. Triplicate or quadruplicate aliquots (2 ml, containing approximately 1 mg of protein) were pipetted into vessels containing the drugs. Following the incubation with drugs, the samples were centrifuged at 2000 × *g* for 1 min at 4° to separate the medium from the slices.

Measurements of PGs and data analysis. The amounts of PGE and PGF released into the medium under different conditions were measured by radioimmunoassay utilizing dextran-charcoal absorption, followed by centrifugation to separate free from bound ligand. An aliquot from the supernatant of each sample was counted in a Packard 2000CA scintillation counter at 69% efficiency. The log-logit transformation was used to convert the per cent-bound values to pg/100 mg of tissue. The displacement values of the samples were all within the linear region of the transformed standard curve (16–250 pg/assay tube). The results are expressed as means ± standard errors for each experiment. Statistical analyses between means of more than two experimental groups were made using one-way analysis of variance followed by Tukey's HSD Test for multiple comparisons. Differences between means were considered significant if $p < 0.05$. Student's *t* test was used to determine the statistical significance between means relative to a control group.

Results

Effect of cannabinoids on PG synthesis in brain slices. In prewashed 0.5-mm-thick cortical slices, THC inhibited the release of PGE and PGF relative to controls in a dose-dependent manner (Fig. 1). The inhibition was significant over concentrations of THC ranging from 0.8 μM to 16 μM. In order to determine the structural specificity of the THC-induced inhibition of PG formation in cerebral cortex, we examined the effects of several of the better characterized psychoactive and nonpsychoactive cannabinoids. For these experiments, we utilized 0.5-mm-thick slices cross-chopped into 0.35 × 0.35 mm sections, allowing for a greater range of cannabinoid concentrations to be tested in a single experiment and for each measurement to be made in triplicate or quadruplicate. Incubation of brain cortex slices for 1 hr with THC and Δ^8-THC over a range of concentrations from 0.2 to 24 μM significantly inhibited PGE formation relative to control levels in a dose-dependent manner (Fig. 2a). Concentrations of Δ^8-THC greater than 2 μM elicited a maximal inhibition of 50 ± 5%, with an IC_{50} of around 0.4 μM. The inhibition values for THC were similar to those of Δ^8-THC. The nonpsychoactive congeners were relatively less effective in inhibiting PGE formation; their inhibition curves were shifted to the right by 1 order of magnitude. Concentrations of cannabidiol less than 4 μM were ineffective in reducing PGE formation. Over the concentration range of 12–36 μM, cannabidiol inhibited PGE formation on the order of 30%. The inactive stereoisomer of THC, (+)-THC, was also considerably less effective in inhibiting PGE formation than either of the psychoactive congeners (Fig. 2a).

Whereas the psychoactive and nonpsychoactive cannabinoids all decreased PGE formation with differing relative potencies, the effects of the two classes of cannabinoids on the formation of PGF were in opposite directions (Fig. 2b). Incu-

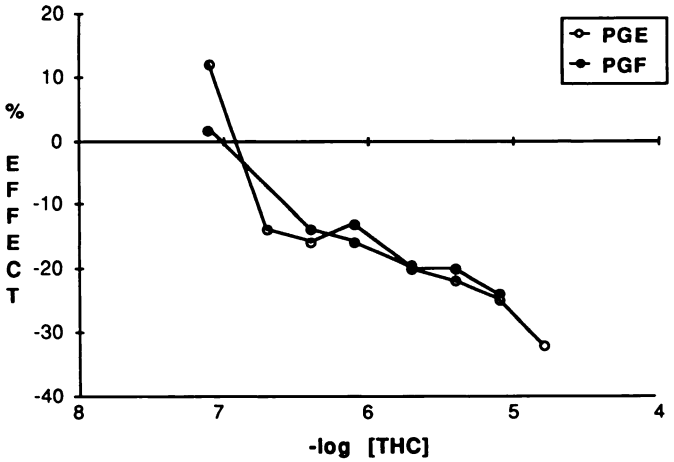

Fig. 1. Effect of THC on PGE and PGF efflux from cortical slices. Cortical slices, 0.5 mm thick, were prepared using a Stadie-Riggs tissue slicer as described under Experimental Procedures. Prewashed slices were incubated in the presence or absence of increasing concentrations of THC for 1 hr at 37°. The amounts of PGE and PGF released into the medium were measured by radioimmunoassay. Values are the means from at least three determinations and are expressed as the percentage increase in PG levels relative to controls. The PGE and PGF levels were, on average, 7.2 ± 1.3 and 1.9 ± 0.4 ng/100 mg of tissue, respectively.

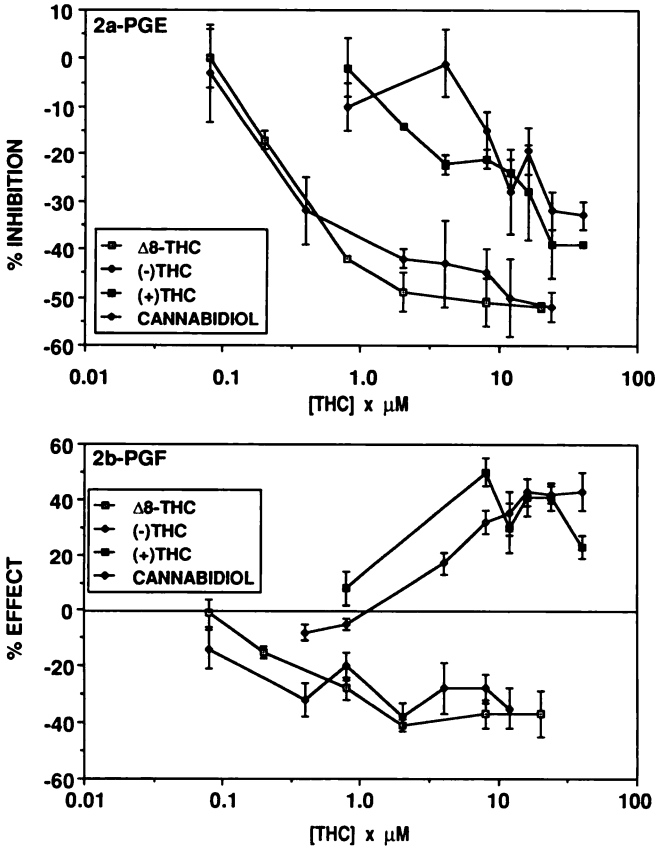

Fig. 2. Dose response curves for different THC congeners on PG efflux from cortical slices. Cortical slices 350 × 350 μm thick were prepared with the aid of a McIlwain tissue chopper and preincubated as described under Experimental Procedures. The washed slices were suspended in KHBS to a final concentration of approximately 1 mg/ml of protein. Two-ml aliquots were incubated in the presence or absence of increasing concentrations of the indicated cannabinoids for 1 hr at 37°. The reaction was stopped by centrifuging the slices at 2000 × *g* for 2 min at 4°. The values are expressed as the percentage increase in PG levels relative to controls and represent the means ± standard errors from at least three experiments, carried out in triplicate or quadruplicate. Control PG levels (mean ± SE/mg of protein) were: PGE, 550 ± 80; PGF 300 ± 40.

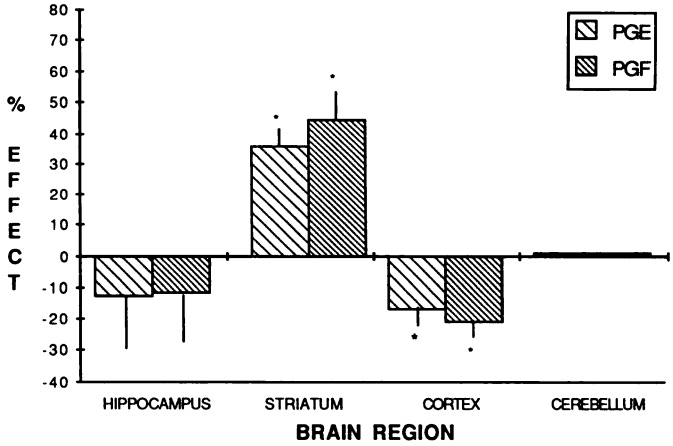

Fig. 3. Effect of THC on PG levels in different brain regions. Slices were prepared from the indicated brain regions as described under Experimental Procedures and incubated in the presence or absence of 0.8 μM THC for 1 hr at 37°. The amounts of PGE and PGF released into the medium were measured by radioimmunoassay. Values are the means ± standard errors from three to six experiments. The control PGE levels (mean ± SE, in ng/100 mg of tissue) were: cortex, 8.3 ± 1.1; cerebellum, 5.0 ± 0.7; hippocampus, 2.9 ± 0.6; and striatum, 1.5 ± 0.3. Control PGF levels were: cortex, 3.1 ± 0.6; cerebellum, 1.8 ± 0.4; hippocampus, 1.4 ± 0.3; and striatum, 1.5 ± 0.3. *, $p < 0.05$, relative to the control values for each region, as determined by Student's two-tailed *t* test.

bation of cerebral cortex slices in the presence of THC or Δ^8-THC inhibited PGF formation with a potency that was less pronounced than that for PGE. In marked contrast to the inhibition of PGF levels by the psychoactive congeners, the nonpsychoactive congeners significantly stimulated PGF formation at concentrations greater than 4 μM (Fig. 2b). The maximal stimulation by cannabidiol was on the order of 40% with an estimated EC_{50} value of 4 μM. The stimulations in PGF levels elicited by increasing concentrations of (+)-THC were similar to those for cannabidiol.

Effect of THC on the synthesis of PGs in different regions of the brain. To determine the effects of THC on PG synthesis in different brain regions, 0.5-mm-thick slices from the hippocampus, striatum, cerebellar cortex, and cerebral cortex were incubated for 1 hr in the presence of 0.8 μM THC. In this series of experiments, the effects of THC were stimulatory, inhibitory, or without effect, depending on the brain region assayed (Fig. 3). The release of both PGE and PGF from cerebral cortex was inhibited on average about 18% ± 6%. There was a small inhibition in PG formation in the hippocampus, but the response was more variable than in cortex and thus was not statistically significant. In slices from the cerebellar cortex, THC did not alter the basal release of PGs. In striatal slices, 0.8 μM THC significantly stimulated the release of both PGE and PGF by 36 ± 5% and by 43 ± 9%, respectively.

Effects of THC on ACh-stimulated PG synthesis. We have found that ACh enhances PG synthesis in cerebral cortex slices and other regions enriched in cholinergic receptors (15). More recently, we have observed that ACh enhances markedly the formation of PGE and PGF in the cerebellar cortex (16). The ACh-stimulated formation of PGE and PGF in cerebral cortex and cerebellum is abolished by low concentrations of tropine (20 μM and 50 nM, respectively, which were the lowest concentrations tested), indicating that muscarinic receptors mediate the enhancement of PG levels in the two regions. We therefore sought to determine the interaction between ACh and THC on PG formation in the cortex and cerebellum. Slices were preincubated with 0.8 μM THC for 5 min, followed by incubation with ACh for 30 min. Table 1 shows the effects of THC on the ACh-stimulated formation of PGE and PGF in cerebral cortex slices. In this series of experiments, 5 mM ACh increased PGE and PGF levels by 38% and 90%, respectively. Over the 35-min incubation period, THC inhibited only slightly the basal release of PGE and PGF. In the presence of 0.8 μM THC, ACh-enhanced PGE formation was completely abolished, whereas ACh-induced stimulation of PGF formation was reduced by 70%. The results clearly demonstrate the inhibition by THC of ACh-stimulated PG efflux from cerebral cortex slices. In the cerebellum, 5 μM ACh increased PGE and PGF levels by 116% and 226%, respectively. Incubation of cerebellar slices with THC alone did not alter PG levels. In marked contrast to the results in the cortex, THC did not significantly alter ACh stimulation of PG levels in the cerebellum (Table 2). The results indicate that the effects of THC on neurotransmitter-stimulated PG synthesis are regionally specific.

TABLE 1

Effects of ACh and THC on PGE and PGF levels in cerebral cortex slices

Slices taken from the parietofrontal cortex were prepared as described under Experimental Procedures and preincubated in the presence or absence of THC for 5 min. ACh was added (in the presence of 0.1 mM eserine), and the slices were further incubated for 30 min. The amounts of PGE and PGF/100 mg of tissue released into the medium were measured by radioimmunoassay. The results are the means from three experiments carried out in duplicate.

Treatment	ng of PGE ± SE	%Δ	ng of PGF ± SE	%Δ
Control	4.8 ± 1.0		2.0 ± 0.1	
5 mM ACh	6.6 ± 0.3	+38[a]	3.8 ± 0.4	+90[b]
0.8 μM THC	3.9 ± 0.3	−19	1.7 ± 0.2	−15
5 mM ACh + 0.8 μM THC	4.4 ± 0.5	−8[c]	2.4 ± 0.3	+20[c]

[a] $p < 0.01$ relative to controls. Significance was calculated by one-way ANOVA.
[b] $p < 0.001$, relative to controls.
[c] $p < 0.05$, relative to ACh.
[c] $p < 0.05$, relative to ACh.

TABLE 2

Effects of ACh and THC on the release of PGE and PGF from cerebellar cortex slices

Slices taken from the cerebellum were prepared as described under Experimental Procedures and preincubated in the presence or absence of THC for 5 min. ACh was added (in the presence of 0.1 mM eserine), and the slices were further incubated for 30 min. The amounts of PGE and PGF/100 mg of tissue released into the medium were measured by radioimmunoassay. The results are the means from five to seven separate experiments.

Treatment	ng of PGE ± SE	%Δ	ng of PGF ± SE	%Δ
Control	5.8 ± 1.2		2.8 ± 0.6	
5 μM ACh	12.5 ± 0.7	+116[a]	9.4 ± 1.6	+226[a]
0.8 μM THC	5.9 ± 0.3	+2	2.9 ± 0.2	+4
5 μM ACh + 0.8 μM THC	10.9 ± 1.2	+88[a]	10.2 ± 0.3	+264[a]

[a] $p < 0.001$, relative to controls. Significance was calculated by one-way ANOVA.

Discussion

The major finding of the present study is that pharmacologically relevant THC concentrations exert complex effects *in vitro* on prostaglandin formation in the brain, i.e., THC stimulates, inhibits, or does not alter basal PG levels in brain slices prepared from different regions. The concentrations of cannabinoids which were used to generate the dose response curves (0.8–24 μM) are pharmacologically relevant since others have shown that, following administration to mice of moderate doses of THC (3–10 mg/kg, intravenously), the membrane concentrations of THC in the cerebral cortex (17, 18) and red blood cells (19) are on the order of 1–20 μM, whereas plasma concentrations are on the order of 0.3–4.5 μM (19). The concentration of THC we used in the regional assays, i.e., 0.8 μM, is within the lower limit of the range of these concentrations.

In the cerebral cortex, all of the cannabinoids inhibited the formation of PGE, although THC and Δ^8-THC, cannabinoids which are psychoactive in man and pharmacologically active in numerous behavioral tests in animals (see Refs. 20 and 21 for reviews), were more potent inhibitors of PGE formation than either the inactive stereoisomer of THC or cannabidiol. The differences between the actions of the two classes of cannabinoids were much more apparent when their effects on PGF formation were compared; the psychoactive cannabinoids decreased, whereas the nonpsychoactive cannabinoids increased, PGF formation. Thus, the effects of cannabinoids on PG synthesis in cerebral cortex slices are structurally specific.

The effects of THC on PG levels were regionally specific. Incubation of cerebral cortex slices with 0.8 μM THC inhibited both the basal and ACh-stimulated formation of PGE and PGF. In contrast, neither the basal nor the ACh-stimulated formation of these PGs in the cerebellum was affected by THC. It may be that distinct muscarinic receptor subtypes with different sensitivities to THC underlie the regional specificity (22). Alternatively, the mechanism of the inhibition may not involve cholinergic mechanisms; rather, the cyclooxygenase enzyme complex, which converts AA to eicosanoids, could be differentially sensitive to inhibition by THC in different brain regions. Measuring of the effect of THC on other cholinergic, receptor-mediated responses, such as phosphoinositide breakdown, would aid in clarifying whether THC acts at the level of the cholinergic receptor or on AA metabolism.

The inhibition of PG formation by THC in cerebral cortex slices contrasts with its stimulation of PG levels in fibroblast cell cultures (10, 11). In striatal slices, however, 0.8 μM THC significantly enhanced the formation of PGE and PGF. The THC-induced increase in PG levels in striatal slices is consistent with the proposal that elevated PG levels are involved in mediating THC-induced catalepsy, as intracranial microinjection of THC and 11-OH-THC into the striatum, but not the globus pallidus, induced dose-dependent catalepsy in rats (23). Furthermore, pretreating mice with NSAIDs, which are potent inhibitors of PG synthesis, abolished the cataleptic effect of THC. Injection of PGE_2 reversed the inhibitory effect of NSAIDs, an observation which suggests that THC-induced catalepsy is dependent on the presence of PGE_2 (12). In another study (24), it was observed that the cataleptic response was attenuated in mice maintained on a diet deficient in essential fatty acids and was restored following administration of AA intraperitoneally or by feeding of a normal diet. Others have reported that PGE_2 significantly potentiated THC-induced catalepsy, analgesia, and anticonvulsant effects, while the PG synthesis inhibitor, diclofenac, antagonized these THC-induced responses (13). The apparent dependence of THC-induced catalepsy on PG levels *in vivo* supports an involvement of PGs in the biochemical mechanisms underlying this effect, the locus of which could involve the striatum.

However, it would be premature to relate cannabinoid-induced changes in PG formation per se to psychoactivity. Agents such as the NSAIDs are more effective than THC in inhibiting PG synthesis (1, 4), yet are not psychoactive at clinically effective doses. If enhanced PG levels were intimately related to psychoactivity, NSAIDs should alter the intensity or quality of the marihuana-induced "high." To our best knowledge, this has not been reported in the scientific literature, although it is possible that NSAIDs may indeed modify THC-induced responses in humans in a subtle way, such that users might attribute the effect to poor quality of drug, inefficient smoking, or other factors. In addition, THC may alter the profile of eicosanoids formed from AA in a manner distinct from that of the NSAIDs, and these effects may act in concert with other THC-induced changes in brain chemistry, e.g., alterations in neurotransmitter metabolism (see Ref. 25 for references), to produce alterations in behavior.

The results of several studies support the proposal that cannabinoids stimulate phospholipase A_2 while they inhibit the activity of cyclooxygenase (26, 27). In platelets, THC increased

unesterified AA levels and altered the profile of eicosanoid formation such that the levels of lipoxygenase-derived products were elevated at the expense of the cyclooxygenase pathway, thus resulting in a net decrease in the percentage of AA that is converted to PGs (9). In lung fibroblasts and other model systems, pretreatment with carboxy-THC, an inactive metabolite of THC, did not affect THC-induced AA release, but inhibited PG formation while stimulating the level of lipoxygenase-like metabolites (28). In synaptosomes and other subcellular fractions of mouse brain, THC increased the release of [^{14}C]AA from prelabeled lipids, although concomitant increases in radiolabeled PGs were not detected (29). The effects of cannabinoids on PG formation in cerebral cortex slices could be interpreted in view of the above findings, i.e., cannabinoids increase the liberation of AA, and THC and Δ^8-THC inhibit the conversion of AA to PGs more effectively than (+)-THC or cannabidiol. The observation that PGF levels are elevated rather than inhibited in the presence of the nonpsychoactive cannabinoids is consistent with the findings by others that PGF synthesis continues in the presence of drugs and other conditions which halt the synthesis of PGE (30, 31).

The manner in which the cannabinoids affect the enzymes of the AA cascade (31, 32) could vary in different tissues. This may be one factor underlying the differing reports regarding the actions of THC on PG formation in different model systems, and may also account in part for the present results in the different brain regions. These issues may be important in elucidating the role of AA and its metabolites in mediating the cellular and behavioral effects of THC and are currently under investigation.

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