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Δ^9-Tetrahydrocannabinol Increases Arachidonic Acid Levels in Guinea Pig Cerebral Cortex Slices

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SUMMARY

Several studies have shown that the major psychoactive component in marihuana, (−)-(*trans*)-Δ^9-tetrahydrocannabinol (THC), increases the level of unesterified arachidonic acid (AA) in non-neural cells in culture. Little is known, however, about the effects of THC on AA metabolism in the mammalian brain. In the present study, slices from guinea pig brain cortex were prelabeled with [^{14}C]AA, and the effects of THC and other cannabinoids on the disposition of esterified and unesterified [^{14}C]AA were measured. Incubation of prelabeled cortical slices with THC rapidly increased free [^{14}C]AA levels in a dose-dependent and saturable manner. A maximal increase of over 4-fold was elicited by 32 μM THC, with the half-maximal response occurring at 8.0 μM. Comparison of the potencies of several other cannabinoids revealed that the inactive stereoisomer of THC [(+)-THC] was equipotent with the naturally occurring isomer in increasing unesterified [^{14}C]AA levels. The relative rank-order of potencies in the cannabinoid series we examined were (−)-THC = (+)-THC > cannabinol > Δ^8-THC > cannabidiol. We also measured cannabinoid-induced changes in the disposition of esterified [^{14}C]AA in the neutral lipids and phospholipids of brain cortex slices. After incubation with 8 μM THC for 1 hr, the radioactivity in triacylglycerols was reduced by over one third. The loss of esterified [^{14}C]AA from triacylglycerols accounted for less than 20% of the THC-induced rise in free [^{14}C]AA; the remainder was accounted for by losses in the radioactivity contained in the phospholipid fraction, particularly from phosphatidylinositol. The loss in radioactivity from phosphatidylinositol alone accounted for over one half of the THC-induced rise in unesterified [^{14}C]AA. The results of the present study indicate that in brain, as in extra-neural cells in culture, cannabinoids increase unesterified AA levels; however, the relative potencies of the cannabinoids we examined in increasing AA levels do not correlate well with their *in vivo* psychoactive potencies.

Marihuana is one of the oldest known psychoactive substances and today is the most widely abused illicit drug in North America (1). It is well established that THC is the principle active component in marihuana. In addition to psychoactivity, cannabinoids possess several potentially therapeutic properties, including antiemetic, analgesic, anti-inflammatory, hypothermic, anticonvulsant, and cardiovascular effects (2). Despite much research, the mechanisms underlying these responses are not well understood. Evidence has been emerging over the past decade that indicates that one of the cellular responses to THC is an elevation in the level of unesterified AA. Burstein and Hunter (3) first showed that THC increased unesterified [^{14}C]AA levels in a dose-dependent manner in HeLa cells previously labeled with [^{14}C]AA. Similar studies in other extra-neural model systems, including murine Leydig cells, human lung fibroblasts, platelets, and pancreatic islets (4–6), have demonstrated that cannabinoids increase cellular levels of unesterified AA. Based upon these and other findings, it was proposed that the activation of phospholipase A_2 mediated wholly or in part the THC-induced increases in free AA.

The mobilization of esterified AA is the obligatory step in the formation of PGs and other eicosanoids, AA-derived metabolites that are potent bioactive compounds. Although the effects of THC on the levels of PGs are complex insofar as they appear to be tissue and dose dependent (7, 8), there is strong indirect evidence supporting the view that eicosanoids are involved in mediating some of the central actions of THC. Nonsteroidal anti-inflammatory drugs, which inhibit PG formation, abolish THC-induced catalepsy (9) and THC resin attenuates the analgesic, hypothermic, and antiepileptic effects of THC in mice (10). Eicosanoids may also be involved in mediating the hypotensive effect of THC, inasmuch as pretreatment with aspirin blocks this response in dogs (11).

Considering the indirect evidence that supports a relationship between cannabinoid actions and increases in AA levels, it is somewhat surprising that nonneural cells in culture and cellular preparations derived from peripheral tissues have been

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ABBREVIATIONS: THC, (−)-(*trans*)-Δ^9-tetrahydrocannabinol; AA, arachidonic acid; BSA, bovine serum albumin; CBD, cannabidiol; CBN, cannabinol; DMSO, dimethyl sulfoxide; DG, diacylglycerol; KHBS, Krebs-Henseleit bicarbonate saline; MG, monoacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; PI, phosphatidylinositol; PS, phosphatidylserine; TG, triacylglycerol.

used almost exclusively to study the effect of THC on AA levels. Although these systems have the advantage of being homogeneous and relatively simpler than the brain, it is not clear whether they adequately represent the central nervous system as models with which to investigate the biochemical mechanisms underlying cannabinoid-induced behavioral effects. The relationship between cannabinoid action and AA levels in neural preparations with intact synapses has hitherto not been explored. In order to study how cannabinoids alter AA metabolism in the brain, we examined how THC and other cannabinoids affect unesterified [^{14}C]AA levels in prelabeled slices from guinea pig brain cortex. Furthermore, to study the mechanisms underlying the cannabinoid-induced changes in unesterified AA levels, we measured changes in the disposition of [^{14}C]arachidonyl residues in the major classes of phospholipids and neutral lipids in brain cortex slices after their exposure to cannabinoids.

Experimental Procedures

Materials. The cannabinoids used in this study, which include THC, its inactive stereoisomer [(+)-THC, which was 95% pure stereochemically], Δ^8-THC, CBN, and CBD were obtained through the National Institute on Drug Abuse (Rockville, MD) from the Research Triangle Institute (Research Triangle Park, NC). They were stored in absolute ethanol at -20° under an inert atmosphere. The purity of the drugs was 98% or greater, as measured by gas chromatography or high pressure liquid chromatography. Just before their use, the ethanol was removed under a stream of N_2, followed by suspension of the residue in DMSO. The [^{14}C]AA (65–80 Ci/mmol) was purchased from NEN Research Products (Boston, MA). All glassware contacting the cannabinoids or [^{14}C]AA was treated with Surfa-Sil (Pierce Chemical Co., Rockford, IL). The BSA (fatty acid depleted) and DMSO were purchased from Sigma Chemical Co. (St. Louis, MO). The thin layer chromatography plates used were 0.25-mm thick silica gel 60 (E. M. Merck, Darmstadt, West Germany), and the solvents were all analytical reagent grade or better.

Tissue preparation and incorporation of [^{14}C]AA. Adult male guinea pigs (400–500 g) were stunned and decapitated. Their brains were rapidly removed and rinsed in ice-cold KHBS (saturated with 5% CO_2 in O_2, to a final pH value of 7.4) containing 10 mM glucose. A modified Stadie-Riggs tissue slicer was used to remove 500-μm thick slices from the cerebral cortex of two guinea pigs. These brain cortex slices were then cross-chopped, in a cold room, into 350 × 350 μm sections using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY). The slices were placed in a 25-ml Ehrlenmeyer flask containing 5 ml of 0.2% BSA in KHBS saturated with 5% CO_2 in O_2, and the flask was tightly stoppered. After a preincubation period of 15 min, the medium was replaced with 5 ml of 0.2% BSA in KHBS containing 5 μCi of [^{14}C]AA, and the slices were incubated for an additional 1 hr. The BSA was used in order to bind excess free [^{14}C]AA during the prelabeling, a procedure that resulted in lowered basal levels of free [^{14}C]AA after washout of the label, as reported by others (12). After the incubation, excess label was removed by rinsing three times with 15 ml of KHBS in 0.2% BSA, followed by incubating the slices twice with 5 ml of the same buffer for 10 min. The BSA was removed by rinsing three times with 15 ml of KHBS, and the slices were suspended in KHBS (without BSA) to a final protein concentration of around 0.8 mg/ml. Two-milliliter aliquots of the suspension were pipetted into Ehrlenmeyer flasks containing the appropriate amounts of THC or the vehicle alone (which was 0.25% DMSO, a concentration that had no effect on the disposition of [^{14}C]AA). The vessels were then flushed with a stream of 5% CO_2 in O_2, tightly stoppered, and incubated for varying times. The reaction was terminated by placing the vessels in a slurry of dry ice in ethanol, immediately after which the tissue lipids were extracted.

Determination of radioactivity in individual lipids. The frozen brain slices were extracted using a modified (the aqueous phase was acidified to pH 3.5) Bligh and Dyer method (13). The total lipid extract was fractionated using two different chromatographic systems. The neutral lipids, comprising MG, DG, TG, and AA, were separated using a one-dimensional system on activated silica gel G plates in hexane/diethyl ether/acetic acid, 65:35:4. In this system, the phospholipids, which remain at the origin, were also removed for scintillation counting. In other experiments, the individual phospholipids were separated by thin layer chromatography using a two-dimensional system. Samples and carrier lipids were applied as 2 cm × 2 mm bands and developed in two dimensions (first dimension, tetrahydrofuran/acetone/methanol/water, 50:20:40:8; second dimension, chloroform/methanol/acetic acid/water, 75:45:12:3). The lipid bands were visualized by brief exposure to I_2 and then scraped from the plate and transferred into scintillation vials. After 10 ml of scintillation fluid was added the radioactivity in the lipids was measured with a Packard 2000CA scintillation counter at 67% efficiency.

Data analysis. Over 95% of the total radioactivity contained in the tissue homogenate was recovered in the lipid extract, with the remainder distributed in the aqueous phase. The radioactivity in the aqueous phase from THC-treated samples did not differ significantly from controls. Essentially all (99% ± 3%) of the radioactivity applied to the plate was recovered in the individual bands taken for scintillation counting. There were no significant differences between the total radioactivity contained in the control and experimental groups, a result that indicated that there was a net balance in the disposition of the [^{14}C]AA. Therefore, the dpm values in the total lipid extracts were in some cases normalized to the average of the applied radioactivity in order to correct for differences in tissue content between samples. All results are expressed as the averages ± standard errors of the mean values from at least three individual experiments carried out in triplicate or quadruplicate, unless otherwise stated in the legend. In many of the experiments, we could not determine the maximal response (nor EC_{50}) values for all of the different congeners, because achieving saturation would have involved using toxic cannabinoid concentrations. Instead, the relative potencies of the congeners were compared at several concentrations using THC as the reference drug. Statistical significance was calculated using Student's t test with $p < 0.05$ for a two-tailed distribution.

Results

Effect of THC on levels of unesterified AA. Incubation of cerebral cortex slices with 8.0 μM THC markedly elevated unesterified [^{14}C]AA levels. The response was time dependent and occurred after incubation with THC for 5 min, at which time unesterified [^{14}C]AA levels were elevated by 40% (Fig. 1). The radioactivity in AA rose in a linear manner up to 20 min, at which time [^{14}C]AA levels were elevated by 140%. A maximal increase of 185% occurred at 1 hr, whereas by 2 hr the rise in free [^{14}C]AA declined to one half of that observed at 1 hr. We therefore routinely examined the effects of THC and its congeners on AA metabolism after an incubation period of 1 hr.

The THC-induced increases in free AA levels were dose dependent and saturable (Fig. 2). The EC_{50} for the response was on the order of 8.0 μM, with a maximal stimulation of 4-fold above control levels occurring at 32 μM THC. The effect of the inactive stereoisomer of THC, (+)-THC, was not statistically different from that of the naturally occurring isomer. Comparison of the potencies of four naturally occurring cannabinoids in increasing free [^{14}C]AA levels revealed that the response was structurally specific (Fig. 2). Cannabinol was 2-fold less potent than THC, and Δ^8-THC was one third as active as THC in increasing [^{14}C]AA levels. The increases

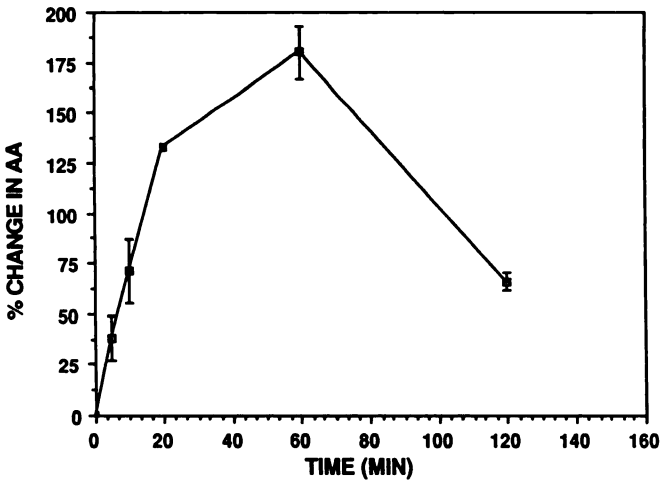

Fig. 1. Time course of THC-induced increases in unesterified [^{14}C]AA. Brain cortex slices were prelabeled with [^{14}C]AA as described under Experimental Procedures. The labeled slices were incubated with or without 8.0 μM (−)-THC for 5–120 min at 37°, after which the radioactivity in the free fatty acids was determined. The results are from at least three experiments and are expressed as the mean percentage increase (± standard error) in the DPM values relative to control samples. The controls were incubated with the vehicle alone (0.25% DMSO).

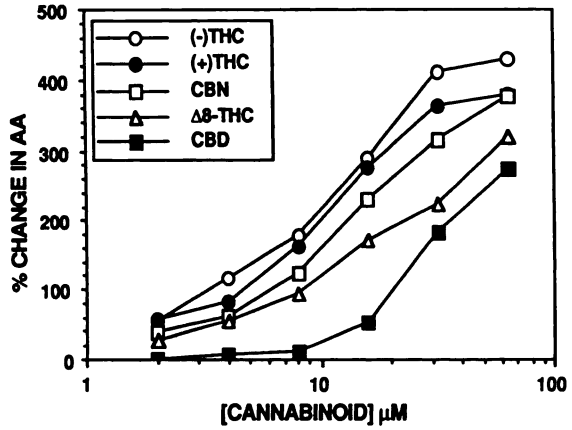

Fig. 2. Dose-response curves of the effects of cannabinoids on unesterified [^{14}C]AA levels. Prelabeled cortical slices were incubated with increasing concentrations of cannabinoids for 1 hr at 37°. After separation by thin layer chromatography, the radioactivity in the free fatty acid fraction from control and THC-treated lipid extracts was measured. The results are from at least three experiments and are expressed as the mean percentage increase in the dpm values relative to control samples (standard error values were within 13% of the mean value).

elicited by CBN, Δ^8-THC, and CBD did not appear to diminish with increasing concentrations as high as 64 μM. We did not attempt to achieve saturation with these less active THC congeners, because higher concentrations of cannabinoids may lead to cellular toxicity (3). The least active of the cannabinoids that we tested was CBD. Over a range of concentrations between 2 and 8 μM, CBD did not significantly affect AA levels compared with control samples, in contrast to the marked increases elicited by similar concentrations of THC. At the higher concentrations, CBD was 4-fold less potent ($0.001 < p < 0.05$) than THC in releasing AA.

Effects of cannabinoids on the disposition of [^{14}C]AA in neutral lipids. In order to gain information on the mechanisms underlying the THC-induced increases in unesterified [^{14}C]AA, cortical slices were incubated with increasing concentrations of THC for 1 hr as above, and the radioactivity contained in the major species of neutral and phospholipids was measured. Figs. 3–5 depict the effects of THC on the disposition of [^{14}C]arachidonyl residues in the DG, TG, and phospholipid fractions, which together comprise essentially all of the esterified stores of [^{14}C]AA in brain cortex lipids.

Concentrations of THC from 2 to 64 μM resulted in graded reductions in [^{14}C]AA levels in TG (Fig. 3). A maximum decrease of 50–60% in the radioactivity contained in TG was observed after incubation of the slices for 1 hr with 32 μM of the different cannabinoids. Comparisons of the EC_{50} values after a 1-hr incubation, at a time when the increases in unesterified [^{14}C]AA were maximal (Fig. 1), revealed no significant differences in the potencies of the cannabinoids we tested. Reductions in TG levels were also observed after shorter incubation times; after exposure to 8 μM cannabinoids for 10 or 20 min, the only significant changes in esterified [^{14}C]AA we observed were reductions in TG levels that equaled the rises in unesterified [^{14}C]AA. At the earlier times as well, the cannabi-

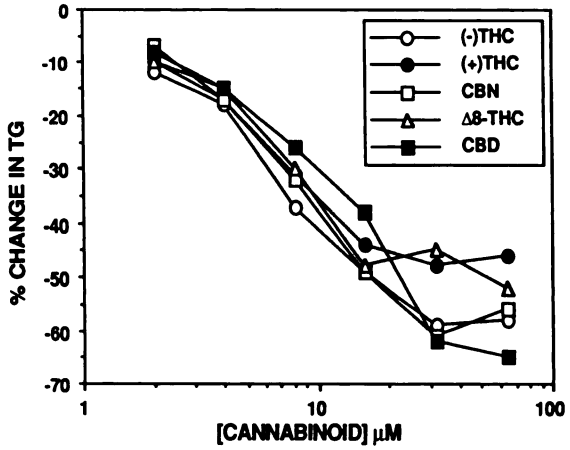

Fig. 3. Dose-response curves of the effects of cannabinoids on [^{14}C]AA levels in TG. Brain cortex slices were prelabeled with [^{14}C]AA and then incubated with and without cannabinoids for 1 hr. The radioactivity contained in the TG band was measured as described. The results are from at least three experiments and are expressed as the mean percentage decrease in the dpm values relative to control samples.

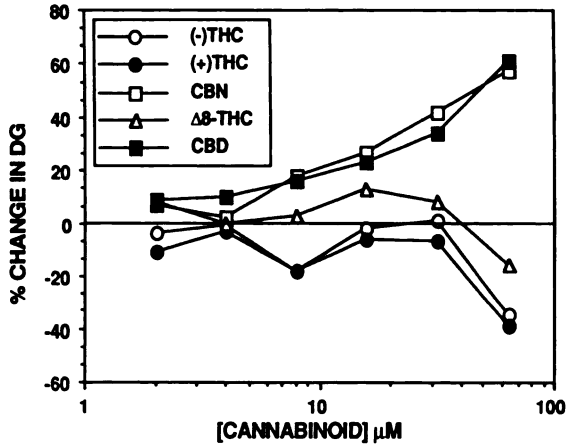

Fig. 4. Effect of THC on esterified [^{14}C]AA levels in DG. Prelabeled cortical slices were incubated with cannabinoids for 1 hr and the radioactivity in the DG fraction was measured as described. The results are from at least three experiments and are expressed as the mean percentage change in the dpm values relative to control samples (standard error values were within 20% of the mean value).

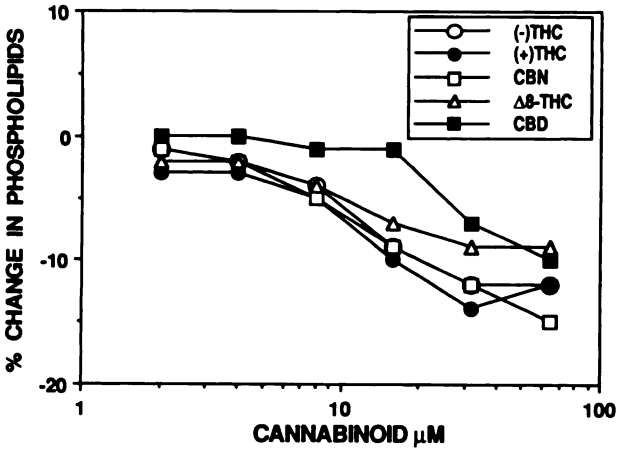

Fig. 5. Effect of cannabinoids on esterified [^{14}C]AA levels in the phospholipid fraction. Prelabeled slices were incubated without and with increasing concentrations of cannabinoids for 1 hr, after which the radioactivity esterified in the total phospholipid fraction was determined. The results are from at least three experiments and are expressed as the mean percentage increase in the dpm values relative to control samples (standard errors were within 14% of mean values).

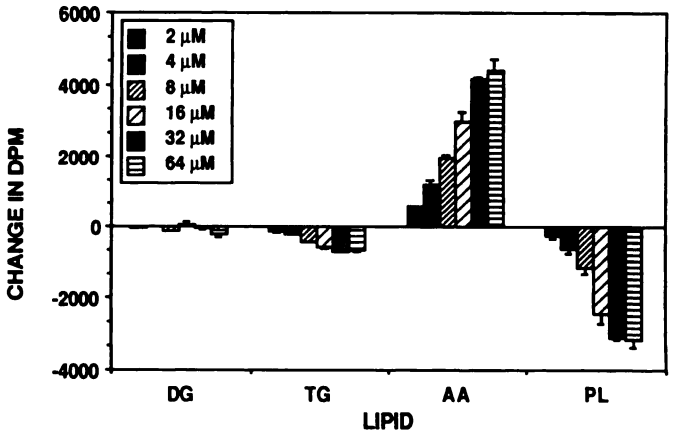

Fig. 6. THC-elicited changes in the disposition of free and esterified [^{14}C]AA. Prelabeled slices were incubated without and with THC for 1 hr. The net balance between the rise in unesterified [^{14}C]AA and the loss of esterified [^{14}C]AA from the neutral lipids and total phospholipid fraction is depicted. The results are expressed as changes in dpm values (± standard error) relative to controls for the indicated lipid fractions and are means from at least three experiments carried out in triplicate or quadruplicate.

noids were equipotent in reducing TG levels (results not shown). Thus, although cannabinoid-elicited reductions in TG contribute to the elevation in unesterified AA levels, the response is not structurally specific.

The cannabinoid-induced changes in the radioactivity contained in DG were clearly structurally specific (Fig. 4). Both CBD and CBN elicited dose-dependent statistically significant increases in the [^{14}C]AA contained in DG compared with control samples. On the other hand, both stereoisomers of THC and Δ^8-THC did not significantly alter DG levels.

Effects of THC on the disposition of [^{14}C]AA in phospholipids. The cannabinoids we tested all decreased [^{14}C]AA levels in the total phospholipid fraction in a concentration-dependent manner (Fig. 5). For the most part, the percentage decreases in the phospholipid fraction elicited by the different cannabinoids paralleled the increases in unesterified [^{14}C]AA (Fig. 2), i.e., the relative potencies of the cannabinoids in reducing the radioactivity in the phospholipid fraction were THC, (+)-THC, CBN, > Δ^8-THC > CBD. The relative magnitude of the decreases in radioactivity contained in the phospholipids was under 15%, over the entire range of drug concentrations. It should be pointed out, however, that the phospholipid fraction contains almost 90% of the total store of esterified [^{14}C]AA contained in the lipid extracts (see Table 1). For example, the total lipid extracts contained on average 30,000 ± 2,000 dpm. Of this amount, the average amount of radioactivity esterified in the phospholipids was 27,000 ± 1,500 dpm, with the remainder of the radioactivity (3,000 ± 150 dpm) distributed in the neutral lipids and unesterified AA. Thus, even small losses in radioactivity from the phospholipids would lead to marked increases in unesterified [^{14}C]AA. Inspection of the changes in the disposition of esterified [^{14}C]AA in response to increasing concentrations of THC shows clearly that the loss in the radioactivity from the neutral lipids contributes only a minor amount to the rise in unesterified [^{14}C]AA (Fig. 6). In contrast, on the order of 80% of the increase in free [^{14}C]AA is accounted for by losses from the phospholipids.

Because the apparent losses in radioactivity from the total phospholipid fraction were similar in magnitude to the increases in unesterified [^{14}C]AA, we fractionated the major classes of phospholipids using two-dimensional thin layer chromatography in order to determine whether individual species of phospholipid were affected differentially by THC. Over the entire range of THC concentrations we examined, there were no consistent changes in PC or PS. The radioactivity in these lipids fluctuated only ±4% from control levels (data not shown). In contrast, the radioactivity in PI, PE, and PA were reduced at all THC concentrations used. Table 1 shows a "balance sheet" of the THC-induced changes in the disposition of radioactivity in the major phospholipid classes. Incubation with 8.0 μM THC released 2399 dpm of [^{14}C]AA, which represents an increase of 2.85-fold above control levels. The radioactivity in TG was significantly reduced by 36%, or −363 dpm, which represents 15% of the rise in [^{14}C]AA. The loss of esterified [^{14}C]AA from PE accounts for 17% of the rise in unesterified [^{14}C]AA. The reduction in radioactivity from PI accounts for almost 60% of the increase in unesterified [^{14}C]AA levels that occurs in response to THC. We did not detect any consistent

TABLE 1

Effect of (−)-THC on the radioactivity in lipids prelabeled with [^{14}C]AA

Prelabeled brain cortex slices were incubated with or without 8.0 μM (−)-THC for 1 hr at 37°. The phospholipids and neutral lipids were separated by thin layer chromatography, and their radioactivity was determined. The results are means ± standard errors from three to five experiments.

Lipid	Control	THC	Δ
		dpm	
PC	6713 ± 56	6552 ± 101	−161
PI	15016 ± 77	13712 ± 213	−1304[a]
PS	1266 ± 44	1272 ± 54	+6
PE	3462 ± 76	3050 ± 37	−412[a]
PA	324 ± 19	300 ± 11	−27
MG	181 ± 6	196 ± 8	+15
DG	761 ± 84	699 ± 34	−62
TG	996 ± 47	637 ± 8	−359[b]
AA	1282 ± 47	3655 ± 295	+2344[b]

[a] $p < 0.05$, relative to controls.
[b] $p < 0.001$, relative to controls.

changes in the radioactivity in PC, even though the radioactivity contained in PC was greater than that contained in any of the other lipids, excluding PI.

Discussion

The present work demonstrates that pharmacologically relevant concentrations of THC rapidly increase unesterified AA levels in neural preparations with intact synapses. The concentrations of THC used in this study can be considered pharmacologically relevant because the EC_{50} for the response, 8 μM, is similar to the concentrations found in plasma and membranes at the time of intoxication (14, 15). The increases were dose dependent and saturable, which might suggest that the response is mediated via binding of THC to a specific receptor. However, the inactive enantiomer of THC was equipotent to the native isomer in stimulating the release of [^{14}C]AA in the brain. Thus, the THC-induced increase in unesterified AA in brain is not stereospecific, a finding that indicates that the response is not receptor mediated. However, the THC-induced increases in unesterified AA levels were structurally specific. The relative rank-order potency of the cannabinoids for increasing unesterified [^{14}C]AA levels was (−)-THC = (+)-THC > CBN > Δ^8-THC > CBD.

The lack of stereoselectivity for the THC-induced rise in unesterified AA in the brain is in agreement with work by others in nonneural cell cultures (16). Also, the effective range of concentrations over which THC elicited graded increases in unesterified [^{14}C]AA in the brain is similar to the dose-response curves observed in HeLa cells (3). However, the finding that in brain slices THC is the most potent of several naturally occurring cannabinoids contrasts with the results obtained in HeLa cells in which CBN and CBD, two cannabinoids that are devoid of psychoactivity, were more potent than THC in releasing AA. These two congeners were also more potent than THC in increasing AA and its metabolites in other extra-neural model systems, such as murine Leydig cells (4), and they were much more potent than THC at mobilizing AA in human lung fibroblasts (17). Thus, there is a closer correspondence in the brain cortex slice model between the potencies of several cannabinoids in releasing AA and their pharmacological potencies, as compared with extra-neural cell cultures. However, the present results do not fit well with the *in vivo* psychoactive potencies of the cannabinoids. Firstly, the behavioral effects of THC in humans and animals are stereospecific (18), whereas the two enantiomers were equipotent in the present study. Furthermore, CBN is considerably less psychoactive than Δ^8-THC, the latter cannabinoid being equipotent with THC in humans and animals (19); in the present study, CBN was more potent than Δ^8-THC. Taken together, these results argue against the notion that elevation of unesterified AA levels alone is a primary mechanism underlying the psychotomimetic effects of cannabinoids. We should point out, however, that we have screened relatively few cannabinoids thus far, and the mechanism underlying cannabinoid-induced AA mobilization in brain remains unclear (as discussed below). Therefore, it would be premature to exclude the involvement of AA in the broad response constellation that comprises cannabinoid pharmacology, especially because some of the behavioral effects of cannabinoids may be a composite of "specific" (receptor-mediated) and "nonspecific" (membrane-perturbant) effects (7, 18). For example, an elevation in AA levels could be necessary but not sufficient to elicit a response such as psychoactivity.

Concomitant with the elevation in unesterified [^{14}C]AA levels, incubation of brain slices with THC resulted in marked, dose dependent, and saturable reductions in the levels of esterified [^{14}C]AA in TG. Comparison of the dose-response profiles of several cannabinoids reveals, however, that this response is structurally nonspecific, i.e., there was no relationship between cannabinoid structure and potency for decreasing TG levels. At the higher concentrations, CBD elicited larger decreases in TG levels than Δ^8-THC and THC. The lack of structural specificity in the cannabinoid-induced reduction of esterified AA in TG, together with the small contribution from the neutral lipids to the rise in unesterified AA levels, suggests that the changes in the neutral lipids play a relatively minor role in mediating the cannabinoid-induced rise in AA levels.

Although the percentage changes in radioactivity in the phospholipids were not as marked as in the neutral lipids, the phospholipid fraction contains almost 90% of the AA esterified in membrane lipids; therefore, even small relative losses of esterified AA from phospholipids will elevate unesterified AA considerably. The radioactivity in PE and PI was significantly lower in THC-treated samples relative to controls. The decreases in these two phospholipids accounted for three fourths of the THC-induced rise in unesterified [^{14}C]AA. The bulk of the loss in radioactivity from the phospholipids was from PI. We did not observe any significant losses in the radioactivity contained in PC over the range of THC concentrations examined in this study, even though PC contained a substantial portion of the total [^{14}C]AA esterified in brain lipids.

The question arises as to possible mechanisms underlying the present findings. The activation of neutral lipid and phospholipid acylhydrolases is one mechanism that can lead to increases in unesterified AA (20). The THC-induced changes in the neutral lipids are consistent with increased neutral lipid lipase activity, although other mechanisms may also be involved (see below). Others have reported that THC increases phospholipase A_2 activity in membrane suspensions prepared from pancreatic islets (6) and mouse brain synaptosomes (21). The proposal was based on the observation that THC increases the hydrolysis of exogenously added PC containing [^{14}C]AA in the C-2 position. The finding in the present work that the [^{14}C]AA content in PC was not affected by THC could be interpreted to mean that a PC-specific phospholipase A_2 is not involved in mediating the rises in AA in brain slices. It may be that THC affects the activity of the enzymes involved in lipid metabolism differently in homogenates than in relatively intact brain preparations *in vitro* or that the PC labeled *in situ* resides in a compartment unaffected by THC. Compartmentalization of substrate may be less of a problem when substrate is introduced into a suspension of membrane fragments.

The activation of phosphoinositidase C, followed by DG lipase action, is now recognized as an important pathway that can elevate unesterified AA levels in some cells (22, 23). For example, incubation of pancreatic minilobules with agonists elicits the breakdown of up to one half of the arachidonyl-enriched PI to DG and inositol phosphates by activation of phosphoinositidase C. The DG is then hydrolyzed by neutral lipases, which liberate AA and other free fatty acids (24). It is unlikely, however, that the decreases in [^{14}C]AA-PI observed in the present study result from activation of phosphoinositi-

dase C, because we did not observe rises in DG or PA, which would accompany PI breakdown by this route (18, 20, 21).

Several studies have shown that cannabinoids inhibit acyltransferase activity in membrane suspensions prepared from lymphocytes, synaptosomes, and pancreas (6, 25). Inhibition of reacylation in brain slices by THC could also account in part for the apparent losses in [^{14}C]AA observed in the neutral lipids and in PI, as others have shown that the turnover of arachidonyl residues in TG and PI in the brain occurs rapidly in comparison with other lipids (26). Further study of the effects of cannabinoids on AA metabolism by employing methods that monitor the incorporation of labeled AA into brain lipids will aid in clarifying whether inhibition of acyltransferase activity is involved in the mechanisms by which cannabinoids increase unesterified AA levels in the brain. Also, the measurement of inositol phosphate formation in the presence of cannabinoids will indicate whether the activation of phosphoinositidase C is a response elicited by cannabinoids.

The mobilization of AA is the obligatory as well as the rate-limiting step in the formation of the PGs and other eicosanoids (27). Despite considerable work (see Refs. 7 and 8 for reviews), a consensus is lacking regarding the role of PGs in cannabinoid actions. Interpreting the available data is somewhat difficult because cannabinoids appear to affect the formation of PGs E and F dissimilarly in different tissues. Some investigators have observed a stimulatory effect, whereas others have reported that THC inhibits PG synthesis. In an earlier study, we found that THC inhibited the formation of PGE and PGF in brain cortex slices in a dose-dependent and saturable manner, whereas THC increased PG levels in the corpus striatum (28). Others have reported that THC inhibits PG formation in neural preparations incubated *in vitro* (29, 30). These observations are consistent with the model proposed by others wherein cannabinoids can stimulate AA release while they inhibit the activity of cyclooxygenase (7, 8). Recent work suggests that the AA released by cannabinoids may be preferentially metabolized to lipoxygenase-derived products at the expense of PG formation (5, 6, 31, 32). In view of the emerging evidence on the importance of lipoxygenase metabolites in the periphery as well as in the brain (33, 34), the manner in which THC alters the profile of AA metabolites in the brain may be an important aspect of cannabinoid pharmacology.

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