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# Effects of Δ⁹-Tetrahydrocannabinol on Lymphocyte and Synaptosomal Lysophosphatidylcholine Acyltransferases *in Vivo*

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SUMMARY

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Δ⁹-Tetrahydrocannabinol was administered to mice intravenously at 15–70 mg/kg. Two hours later the effect of the drug *in vivo* on brain synaptosomes and spleen lymphocytes was determined. Inhibition of acyl-CoA–lysophosphatidylcholine acyltransferase was significant in the synaptosome (maximum inhibition, 44%) and lymphocyte preparations (maximum inhibition, 24%). In lymphocytes there was concomitant inhibition of blastogenesis as measured by [³H]thymidine incorporation.

## INTRODUCTION

Studies on acyl-CoA–lysophosphatidylcholine acyltransferase (EC 2.3.1.23) in lymphocytes, where it is localized mainly in the plasma membrane (1), have shown that it can be stimulated *in vitro* by nonspecific mitogens such as phytohemagglutinin and concanavalin A (2). The activation of acyl-CoA–lysophosphatidylcholine acyltransferase apparently leads to a higher steady-state level of polyenoic fatty acids in the membrane, thereby increasing membrane fluidity. As the mitogens are potent initiators of blast transformation, it has been suggested that LPC[1] acyltransferase might play a role in the early cell-mediated immune response (3). In this study we show that lymphocyte blastogenesis and LPC acyltransferase activity can be stimulated in mice following the intravenous administration of concanavalin A *in vivo*. Δ⁹-Tetrahydrocannabinol has been shown to inhibit lymphocyte blastogenesis *in vitro* (4). This effect has been implicated in the reduced levels of cell-mediated immune response seen in some chronic marijuana users (4), although these results have been challenged by other workers (5, 6). In addition, Δ⁹-THC inhibits LPC acyltransferase activity in mouse lymphocytes *in vitro* with a high degree of specificity and at a low concentration (0.35 μM) for half-maximal inhibition (7).

In this study we describe the inhibition of LPC acyltransferase in spleen lymphocytes by Δ⁹-THC administered *in vivo*. Since the degree of inhibition of LPC acyltransferase by cannabinoids *in vitro* appears to be related to the psychoactive potency of these compounds (8), we have also examined the inhibition of brain synaptosomal LPC acyltransferase in mice given Δ⁹-THC *in vivo*. It is known that synaptosomes retain higher levels of radioactivity from labeled Δ⁹-THC than do other brain particulate fractions (9). Here

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[1] The abbreviations used are: LPC, lysophosphatidylcholine; Δ⁹-THC, Δ⁹-tetrahydrocannabinol; TCA, trichloracetic acid.





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we show that mouse brain synaptosomal LPC acyltransferase is substantially inhibited following the intravenous administration of $\Delta^9$-THC at doses of 15–70 mg/kg.

## METHODS

Groups of five Swift-Webster male mice, each weighing 35 g, were given $\Delta^9$-THC in 0.05 ml of ethanol by tail vein injection at dose levels of 15, 25, 30, 50, and 70 mg/kg. Control groups of five animals were injected with 0.05 ml of ethanol only and showed no effects compared with uninjected control groups, as confirmed by other workers (10, 11). Mice were killed 2 hr after injection of $\Delta^9$-THC, since our preliminary studies showed maximal [$^{14}$C]$\Delta^9$-THC uptake into the lymphocyte membrane at this time (Fig. 1). The spleens from each group of mice were removed, pooled, and homogenized in Hanks' balanced salt solution, using a Teflon-glass homogenizer. The suspension was filtered through a small amount (0.3–0.4 g) of glass wool to remove debris. The filtrate was layered on Ficoll-Paque, and lymphocytes were separated by centrifugation at 440 × *g* for 45 min. The interface was removed, washed in balanced salt solution twice, and resuspended in Hanks' minimal essential medium. Cell viability was determined using the trypan blue exclusion test. Then LPC acyltransferase was assayed and 36-hr cell cultures were initiated in order to determine DNA synthesis. In the concanavalin A experiments mice were injected intravenously with 100 $\mu$g of concanavalin A 3 days before death. Lymphocytes were separated according to Folch and Waksman (12) in order to obtain suppressor cell-free nonadherent lymphocytes and whole spleen lymphocytes. Further assays were carried out as described below.

*Determination of [$^{14}$C]$\Delta^9$-THC uptake.* Sixteen mice in five groups were injected via the tail vein with 1 $\mu$Ci of [$^{14}$C]$\Delta^9$-THC in 0.05 ml of ethanol solution (specific activity, 32 mCi/mmole). Animals were killed 0.1, 1, 2, 4, and 16 hr after injection, and their spleens were removed and extracted into 12 ml of balanced salt solution. After filtration and centrifugation, the pellet was resuspended in 5 ml of minimal essential medium, and cell numbers and viability were determined using the trypan blue exclusion test. Lymphocytes were incubated at 37° for 20 min in order to simulate conditions used in the LPC acyltransferase assay and were then precipitated with 2 ml of cold 5% TCA per tube. The mixture was centrifuged at 1000 × *g* for 5 min, and the pellet lipids were extracted as described below. Chloroform-soluble radioactivity was measured, after the removal of solvent by evaporation under nitrogen, by the addition of 5 ml of scintillation fluid composed of 0.3% (w/v) 2,5-diphenyloxazole and 25% (v/v) Triton X-114 in xylene (Anderson's). The samples were counted in a Beckman LS-255 scintillation counter, using automatic quench correction, at an efficiency of 84–88%. Aliquots of the methanolic, water-soluble extract were counted after the addition of Anderson's solution. The percentage of recovered radioactivity that was due to unchanged [$^{14}$C]$\Delta^9$-THC was determined by chromatography of the chloroform extract, to which unlabeled $\Delta^9$-THC was added as a carrier, on thin layers of silica gel G. The developing solvent was toluene, and the resultant $\Delta^9$-THC spot was eluted and counted as described below.

*LPC acyltransferase assay.* Lymphocyte preparations containing $10^7$ cells/ml of minimal essential medium were incubated in triplicate at 37° for 20 min. Then 100 nmoles of oleoyl-CoA and 200 nmoles of lyso[$^{32}$P]phosphatidylcholine in a volume of 0.1 ml were added to the 1-ml lymphocyte suspensions, and incubation was continued for 15 min, after which the reaction was stopped with 3 ml of cold 5% TCA. Samples were centrifuged for 20 min at 1000 × *g*, and the pellet was subjected to lipid extraction with chloroform–methanol–water (1:1:0.4, v/v/v). Separation of lipid was carried out on silica gel 7G (0.25 mm thick) in chloroform–methanol–water (65:25:4, v/v/v). The individual fractions were located by iodine vapor staining, scraped, and counted in a liquid scintillation counter.

*DNA synthesis measured by thymidine uptake.* Two to five replicate cell cultures were incubated in silanized glass vials.

Each sample contained $2 \times 10^6$ cells in 1 ml of minimal essential medium containing 10% fetal calf serum. The incubation was carried out at 37° in an atmosphere of 5% $CO_2$ and 95% air. After 36 hr of incubation, 1 $\mu$Ci of [6-$^3$H]thymidine was added to each tube, and incubation was continued for another 16 hr. DNA was precipitated with 3 ml of 5% TCA and was sedimented at $1000 \times g$ for 30 min. The pellet was washed twice in 5% TCA, then dissolved in 0.5 ml of "NCS" tissue solubilizer and measured in a liquid scintillation counter.

*Determining LPC acyltransferase activity in brain synaptosomes.* Separation of synaptosomes was carried out according to Cotman (13). Synaptosomes were diluted in 0.095 M phosphate buffer (pH 7.4) to give less than 1.0 mg of protein per milliliter of solution, and 1-ml aliquots were used to measure LPC acyltransferase activity as described above.

*Materials.* Cell culture media were obtained from Grand Island Biological Company, and Ficoll-Paque and other chemicals, from Sigma Chemical Company. $\Delta^9$-THC and [$^{14}$C]$\Delta^9$-THC were kindly provided by Health and Welfare Canada. Other radioactive compounds and tissue solubilizer were obtained from New England Nuclear, except for lyso[$^{32}$P]phosphatidylcholine, which was prepared as described previously (14).

## RESULTS AND DISCUSSION

In order to establish that lymphocyte transformation can be stimulated *in vivo* in Swift-Webster mice, as shown for other strains (14), we injected mice intravenously with concanavalin A 3 days before death and measured [$^3$H]thymidine uptake and LPC acyltransferase activity in lymphocytes. Blastogenesis was stimulated by 17 ± 4% ($n$ = 4) in whole spleen lymphocytes and by 61 ± 15% ($n$ = 3) in nonadherent lymphocytes. The stimulation of LPC acyltransferase activity was 12 ± 3% ($n$ = 3) in whole spleen lymphocytes. The stimulation of LPC acyltransferase activity in whole spleen lymphocytes was less than that seen *in vitro* (1, 7), but higher stimulation was observed in nonadherent lymphocytes, which were relatively free from suppressor cells. Other workers have pointed out the importance of splenic suppressor cells (15, 16) and the effect of the age of the animals used (12) on the observed blastogenesis induced by lectins.

In order to find a suitable time point for measuring $\Delta^9$-THC action on lymphocytes, we determined the time course of uptake of $\Delta^9$-THC by the lymphocytes. Using [$^{14}$C]$\Delta^9$-THC (101 $\mu$Ci/mg) in doses of 1 $\mu$Ci/mouse, we measured the uptake of labeled $\Delta^9$-THC by the spleen and by splenic lymphocytes. The two curves were similar, with maxima at 2 hr (Fig. 1). Thin-layer chromatography showed that the radioactivity recovered at 4 hr represented 19% unchanged $\Delta^9$-THC, the remainder being metabolites. On the basis of these experiments and the work of Klausner and Dingell (17) on the uptake of $\Delta^9$-THC by brain and spleen, we estimate the concentration of $\Delta^9$-THC in mouse lymphocytes and brain synaptosomes following a single intravenous dose of 50 mg/kg of $\Delta^9$-THC to be 0.10 nmole/ml ($10^7$ cells) for lymphocytes and 0.15 nmole/ml for brain synaptosomes (containing 0.5 mg of protein per milliliter). These levels are of the same magnitude as those used previously *in vitro*, when the half-maximal concentration ($K_i$) for the inhibition of LPC acyltransferase by $\Delta^9$-

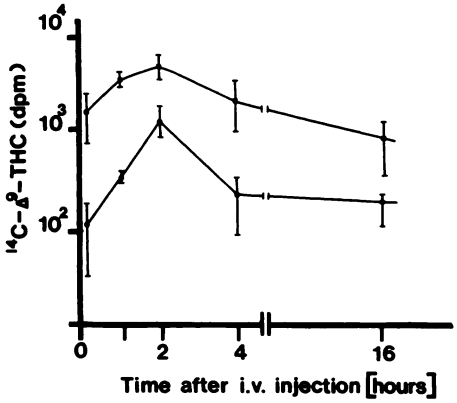


FIG. 1. *[$^{14}$C]$\Delta^9$-THC uptake by spleen* (■) *and spleen lymphocytes* (●)

Each value represents the mean uptake for five animals ± standard deviation.

THC in lymphocytes was found to be 0.35 nmole/ml (7). The levels found in lymphocytes are in good agreement with those found by other workers (18, 19). Our studies indicate that the concentration of Δ⁹-THC in whole brain 2 hr after a single intravenous dose of 50 mg/kg is 51 nmoles/g, a value that agrees well with a previous estimate of 48 nmoles/g (17). On the basis of the results of the [¹⁴C]Δ⁹-THC uptake studies, we chose to kill the animals 2 hr after intravenous injection with the drug.

Inhibition of blastogenesis *in vivo* by Δ⁹-THC as measured by [³H]thymidine incorporation into lymphocyte DNA is shown in Fig. 2. All doses, with the exception of the 15 mg/kg dose, produced statistically significant inhibition ($p < 0.05$) of [³H]thymidine uptake. The highest inhibition obtained at 50 mg/kg was 59%. By analogy with our experiments *in vitro*, we estimate that the Δ⁹-THC concentration in the lymphocyte membrane over the dose range of 15–50 mg/kg is equivalent to a concentration in the lymphocyte suspension *in vitro* of 0.04–0.2 μM. Carchman *et al.* (20) observed inhibition of DNA synthesis in Lewis lung tumor cells at a Δ⁹-THC concentration of 4 μM. Similarly, Blevins and Regan (21) reported inhibition

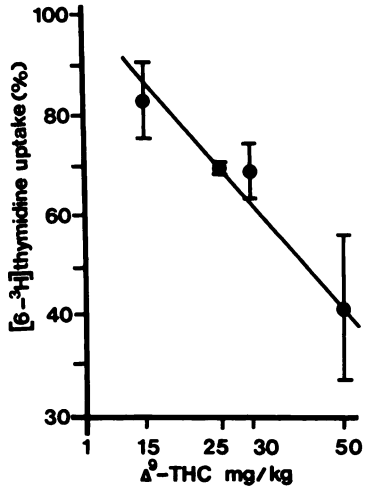


FIG. 2. *Inhibition of blastogenesis as measured by incorporation of [6-³H]thymidine into lymphocyte DNA*

Values are expressed as percentages of controls and are given as means ± standard deviations.

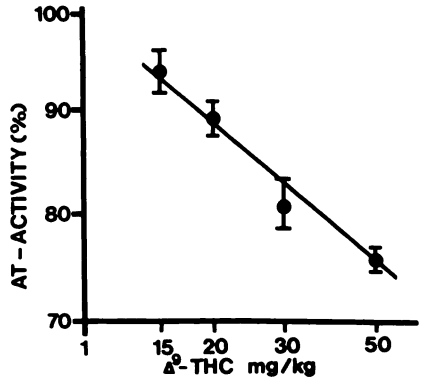


FIG. 3. *Inhibition of lymphocyte LPC acyltransferase (AT) activity*

Values are expressed as percentages of controls and are given as means ± standard deviations.

of [³H]thymidine uptake in fibroblasts as well as in human and mouse neuroblastoma cells of the order of 11–17%, using 0.32 μM Δ⁹-THC.

Figure 3 shows that concomitant with the inhibition of lymphocyte blastogenesis by Δ⁹-THC *in vivo* there was a decrease in lymphocyte LPC acyltransferase activity. This inhibition was proportional to the dose of Δ⁹-THC and was significant ($p < 0.05$) at all concentrations above 15 mg/kg. Ferber and Resch (1) proposed that activation of LPC acyltransferase by mitogens increases membrane fluidity by increasing the polyenoic fatty acid content of the phospholipid bilayer. Since this activation takes place within 30 min *in vitro*, they proposed that these membrane changes are an early event in the cell-mediated immune response. Whether or not the inhibition of LPC acyltransferase is causally related to the later changes in macromolecular synthesis, the data suggest that the process of transformation is interfered with at a very early stage following lectin stimulation. The inhibition that has been described for the lymphocyte enzyme *in vitro* (7) is attainable *in vivo* at concentrations of Δ⁹-THC which we estimate are of the same order as those giving similar inhibition *in vitro*. The experiments *in vitro* indicated that the LPC acyltransferase inhibition was not caused by displacement of concanavalin A from the lymphocyte membrane (7) and that

the effect was not reversible by increased levels of substrates. The enzyme inhibition by $\Delta^9$-THC which had been shown in lymphocytes *in vitro* and *in vivo* could also be seen in mouse brain synaptosomes following a single intravenous dose of $\Delta^9$-THC *in vivo* (Fig. 4). LPC acyltransferase was inhibited to a similar extent in the synaptosomal preparations; for example, at the 40 mg/kg dose the inhibition was 21% in synaptosomes and 20% in splenic lymphocytes. Studies in progress in our laboratory on the inhibition of LPC acyltransferase by $\Delta^9$-THC in mouse brain synaptosome preparations *in vitro* indicate that the concentration for 50% inhibition of the enzyme ($K_i$) in synaptosomes (0.30 $\mu$M) is very similar to that previously reported for lymphocytes (0.35 $\mu$M). Other workers have shown inhibition of membrane-bound synaptosomal enzymes by $\Delta^9$-THC *in vitro*. ($Na^+ + K^+$)-ATPase EC 3.6.1.3) is inhibited *in vitro* by $\Delta^9$-THC at a $K_i$ of 10 $\mu$M, and $Mg^{2+}$-ATPase is inhibited at a $K_i$ of 25–30 $\mu$M (22). More work is necessary to determine whether inhibition of these membrane enzymes by $\Delta^9$-THC shows the same specificity for psychoactive cannabinoids as we have demonstrated for inhibition of lymphocyte LPC acyltransferase *in vitro* (8). The $K_i$ for the inhibition of LPC acyltransferase is about 100 times lower than that for the ($Na^+ + K^+$)-ATPase, but it is known that the presence of serum proteins with a high capacity for the competitive binding of lipids can affect $K_i$ values profoundly, and no conclusions should be drawn from these values at this time.

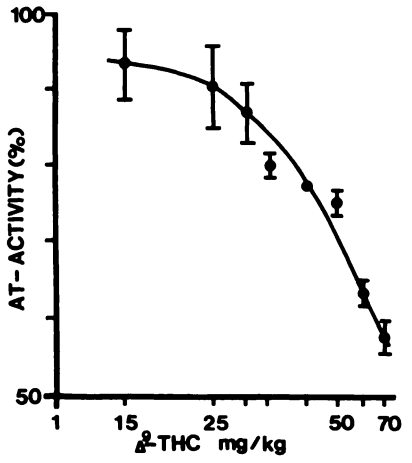


FIG. 4. *Inhibition of LPC acyltransferase (AT) by $\Delta^9$-THC in synaptosomes*

Values are expressed as percentages of controls and are given as means ± standard deviations.

While it is tempting to speculate on the effect that inhibition of the synaptosomal enzyme by $\Delta^9$-THC might have on neurotransmission across the synapse, such speculation is premature. It is by no means clear what role LPC acyltransferase may play in maintaining membrane structure. Further work is needed to determine whether the inhibition of lymphocyte and synaptosomal LPC acyltransferases has any significance for the pharmacological actions of cannabinoids.

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