THE EFFECT OF Δ^9-TETRAHYDROCANNABINOL ON CYTOPLASMIC DNA METABOLISM

Henrietta Margolis-Kazan and John Blamire

Department of Biology,
Brooklyn College, CUNY, Brooklyn, New York 11210

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SUMMARY. Tetrahydrocannabinol has been shown to interfere with DNA synthesis in a variety of organisms and cell systems. Results presented in this paper show that in Volvox species, strain HK-10, Δ^9-tetrahydrocannabinol acts specifically on cytoplasmic DNA, by preventing its synthesis and mediating in its degradation. CsCl density gradient centrifugation profiles of DNA from Volvox, labeled with [^{3}H]-adenine in either the presence or absence of Δ^9-tetrahydrocannabinol are presented. This data demonstrates that while nuclear DNA continues to be synthesized in the presence of the drug, the incorporation of label into the cytoplasmic DNA species is halted. In addition, Volvox DNA prelabeled with [^{3}H]-adenine, and later exposed to Δ^9-tetrahydrocannabinol in vivo, shows that the drug also mediates in the degradation of pre-existing cytoplasmic DNA.

INTRODUCTION

The widespread use of marihuana has stimulated much interest in research on the mode of action and effects of its major psychoactive ingredient Δ^9-THC.[1] Reports of changes in DNA synthesis in cultured human lung cells after exposure to marihuana smoke (1) and chromosomal breakage in chronic marihuana users (2) have led to futher studies on the interactions between cannabinoids and DNA. THC has been shown to interfere with macromolecular synthesis in Tetrahymena (3), and, more recently, in studies related to is antineoplastic activity in vivo (4), Δ^9-THC was shown to inhibit DNA synthesis in Lewis lung (adenocarcinoma) cells in tissue culture (5) without decreasing precursor uptake (6).

In this study we report a specificity in the interaction of Δ^9-THC with the DNA metabolism of Volvox. In this simple, multicellular organism, often used as a model system for studies on development (see Discussion), THC acts almost exclusively on cytoplasmic DNA. In our studies, the

[1] The abbreviations used are: Δ^9-THC, 1-Δ^9-trans-tetrahydrocannabinol; THC, trans-tetrahydrocannabinol; TCA, trichloroacetic acid; Tween 40/80, Polyoxyethylene Sorbitan monopalmitate/Polyoxyethylene Sorbitan monooleate; SVM, Synthetic Volvox Medium.

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synthesis of this DNA species is stopped upon addition of the drug, and with time, existing cytoplasmic DNA is degraded. Volvox's nuclear DNA metabolism, however, remains virtually unaffected by Δ^9-THC during the same period.

MATERIALS AND METHODS

1. Growth and radioactive labeling of Volvox. The strain of Volvox used this study was V. carteri f. nagariensis (HK-10, female). Axenic stocks were maintained at about 25°C in 15 ml screw cap tubes containing 5 or 10 ml of SVM (8) at either pH 7.0 or pH 8.0 (9) at a light intensity of 300 ft candles (16 hr light, 8 hr dark).

Heterogeneous growth conditions consisted of transferring aliquots of stock cultures to 5 ml SVM (pH 7.0) in a 20 x 150 ml sterile tube and gently shaking on an orbital shaker at 400 ft candles cool white fluorescent light (16/8, 25-30°C).

The DNA of Volvox was labeled with 2-[^{3}H]adenine (New England Nuclear) at concentrations ranging from 20 uCi to 30uCi/ml in SVM. The usual labeling time was 18 hr (0.25 generations) unless otherwise noted, and all radioactivity incorporated into DNA was monitored by cold TCA precipitation of hot alkali stable counts onto Whatman GF/A (2.4 cm) filters, washing with 5% TCA, 95% ethanol and drying. Each dried filter was then transferred to 4 ml toluene based scintillant (Liquorfluor, New England Nuclear) and counted in a Nuclear Chicago Liquid Scintillation Spectrometer.

2. Preparation of lysates. Small radioactive cultures were dissociated and cell lysates prepared, after checking for bacterial contamination, by the methods described by Margolis-Kazan and Blamire (7). Spheroids were dissociated by the addition of 100 ug/ml Protease (Sigma Type VI) and incubation for 15-45 min at 36°C. Dissociated cells were harvested by centrifugation at 4,000 x g for 10 min, washed twice with saline EDTA (0.15M NaCl, 0.1 Methylenediaminetetraacetate pH 8.0) and resuspended in 0.7 ml saline EDTA. Two to four drops of 30% Sarkosyl NL-97 (Ciba-Geigy Co.) were added (approximately 5-10% final concentration) and the lysates heated to 60°C for 30-60 min before storing at -20°C until required.

3. Preparative CsCl density gradients. These were carried out on all lysates by methods already published (7). Each sample was centrifuged at 33,000 rpm in a Spinco 50 Ti rotor for 48-65 hr at 19°C. Twenty drop fractions were collected from the bottom of each tube (30 fractions) and the bands of radioactive DNA assayed as described above. The identity of each DNA species was determined from its positions relative to that of Escherichia coli DNA (1.710 gm/cm^3) included in the same gradient as a reference marker.

4. Use of Δ^9-THC. Δ^9-THC was supplied as a solution (20 mg/ml) in 95% ethanol by DHEW, Public Health Service, National Institute of Drug Abuse. Stock solutions were prepared by transferring 1 ml of the ethanol solution to 1 ml Tween 40/80, thus creating a final solution of 10 mg/ml THC, 45-50% ethanol, 25% Tween 40 and 25% Tween 80. A solution of 50% ethanol, 25% Tween 40 and 25% Tween 80 (termed "carrier" hereafter) was also prepared and used in all control experiments.

RESULTS

The effect of THC on DNA synthesis in Volvox. We have previously shown that differential DNA synthesis in Volvox can be monitored using the incorporation of 2-[^{3}H]-adenine into DNA molecules, which are then resolved into nuclear and cytoplasmic species by their positions relative to marker E. coli DNA in preparative CsCl gradients (7) (see Fig. 1, legend).

Δ^9-THC is not soluble in water, therefore, in order to test its effects on DNA synthesis in Volvox, a suitable "carrier" (see Materials and Methods) had to be used as a vehicle to suspend the THC in the SVM media. All drug treated cultures, therefore, contained this carrier, and hence the effects of equal concentrations of carrier alone upon [^{3}H]-adenine incorporation into Volvox DNA had to be elucidated. It can be seen from Fig. 1 that CsCl profiles of DNA taken from heterogeneously grown Volvox spheroids incubated in SVM containing 20 uCi/ml [^{3}H]-adenine for 18 hr in the absence (Fig. 1A) or presence (Fig. 1B) of carrier (40 ul/ml), dissociated and lysed as described in Materials and Methods, are similar in amounts and proportions of [^{3}H]-adenine incorporation into the nuclear and cytoplasmic DNA. Also, since the resolution of the two DNA species is improved and the background lowered when Volvox are grown in the presence of carrier (presumably due to improved elimination of the glycoprotein matrix of Volvox spheroids (unpublished observations)), other controls presented or discussed contain concentrations of carrier equivalent to those present in the drug treated cultures.

Fig. 1C shows the effect of Δ^9-THC upon DNA synthesis in Volvox. Volvox cultures (5 ml in SVM) to which [^{3}H]-adenine and Δ^9-THC (400 ug/ml) had been added simultaneously, were incubated for 18 hr, after which the spheroids were dissociated and lysed as above. The CsCl profile (Fig. 1C) shows that only one DNA species is present as compared to the two seen in the carrier control (Fig. 1B). The banding position of

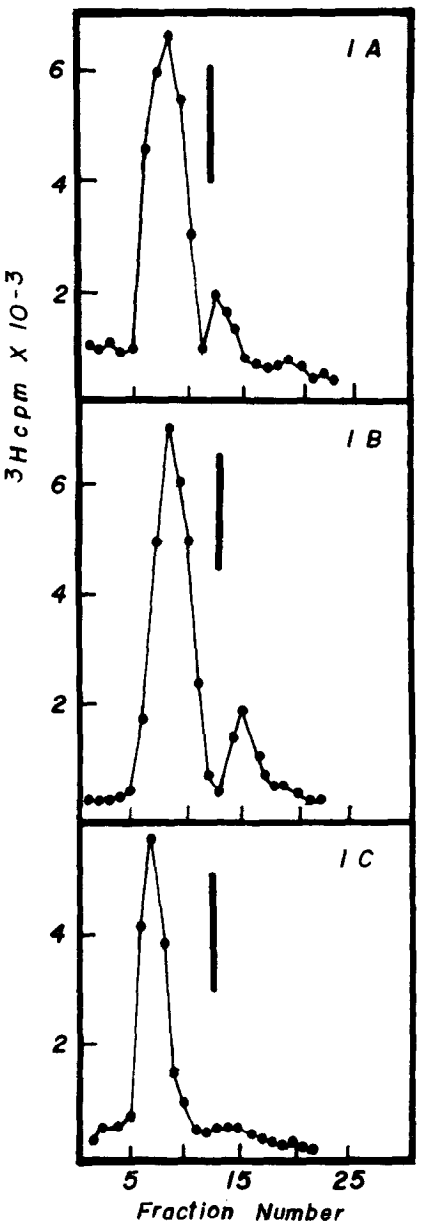

Figure 1. DNA synthesis in the presence or absence of THC and carrier: Preparative CsCl density gradients of radioactively labeled DNA prepared as described in text, with solid circles representing cpm [^{3}H] Volvox DNA (nuclear DNAρ-1.715 g/cm^3; cytoplasmic DNAρ-1.705 g/cm^3) (7) and vertical bars indicating the banding position of [^{14}C] E. coli DNA (ρ-1.710) included in the same gradient as a marker.

A. Control experiment, no carrier, no THC.
B. Carrier (40 ul/ml) control.
C. THC experimental (400 ug/ml).

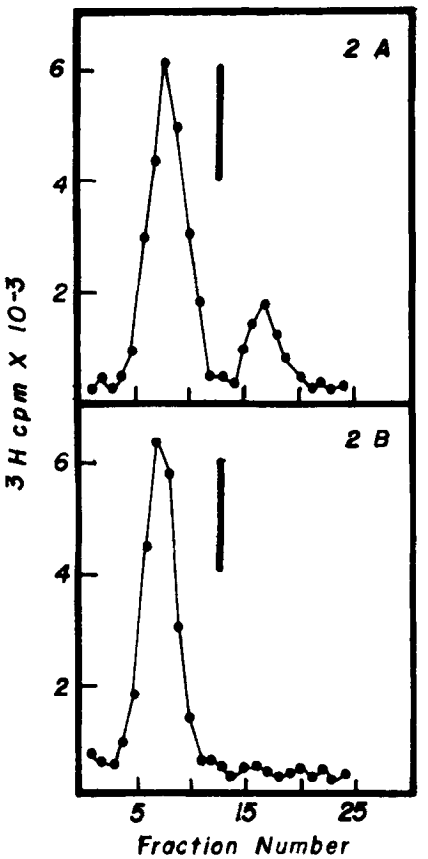

Figure 2. Degradation of prelabeled DNA by THC: Preparative CsCl density gradients of radioactively prelabeled DNA, prepared and treated as described in text, with solid circles representing [^{3}H] Volvox DNA, and vertical bars indicating the banding position of [^{14}C] E. coli marker DNA.
A. Control experiment , (30 ul/ml) carrier.
B. THC experimental (300 ug/ml).

this DNA relative to the [^{14}C] E. coli DNA density marker indicates that only nuclear DNA is synthesized in the presence of Δ^{9}-THC (400 ug/ml). Concentrations of THC at or below 200 ug/ml appear to have little or no effect on nuclear or cytoplasmic DNA in Volvox, thus most of our studies with the drug have been done at THC concentrations between 300 and 400 ug/ml where the specific effect on cytoplasmic DNA alone has been noted. Higher drug concentrations appear to inhibit nuclear DNA synthesis and cause spheroid death. (Results of concentration studies to be published elsewhere).

The effect of THC on prelabeled DNA in Volvox. The effect of THC on prelabled DNA in Volvox has also been examined. Heterogeneous Volvox cultures were labeled with [^{3}H]-adenine for a minimum of 4-5 days (1-2 generations) under optimum conditions as follows. 5 ml cultures of Volvox spheroids in SVM were first grown in the presence of 20 uCi/ml [^{3}H]-adenine for 48 hr. These cultures were then diluted 50% by transferring 2.5 ml of each aliquot into new tubes containing 2.5 ml of SVM. Additional [^{3}H]- adenine (10 uCi/ml) was added to each of the new 5 ml cultures and after a minimum of 48 hr, most media containing [^{3}H]-adenine was removed and the labeled spheroids resuspended in SVM and brought to a final volume of 10 ml. Carrier (30 or 40 ul/ml) or Δ^9-THC (300 or 400 ug/ml) was added to each tube of prelabeled spheroids and the cultures allowed to incubate for 18 hr. They were then dissociated, lysed and treated as described above.

Fig. 2 shows the results of such an experiment when prelabeled spheroids were treated overnight (18 hr) with 30 ul/ml carrier or 300 ug/ml THC, and their DNA banded in CsCl. In Fig. 2A (carrier-treated prelabeled Volvox) one can easily see that both nuclear and cytoplasmic DNA species are present in ratios similar to those seen in the 18 hr DNA synthesis gradients (Fig. 1A and 1B). The THC treated profile (Fig. 2B), however, contains only one DNA band which can again be identified as nuclear. Since cytoplasmic DNA had been present before exposure to the drug, (as can be seen in the carrier control, Fig. 2A) and untreated control (not presented) we assume that the treatment with THC mediated in the degradation and disappearance of this DNA species from the profile. We intend to confirm this hypothesis with analytical CsCl profiles of unlabeled Volvox DNA before and after treatment with carrier and THC.

DISCUSSION

The multicellular, spheroidal, green flagellate Volvox is rapidly becoming a model system for molecular studies of development and differentiation into two cell types (somatic and reproductive) (7,9,10,11,

12). In our studies on DNA synthesis during the life cycle of this organism (7), we have shown that most cytoplasmic DNA is synthesized only during one particular period in the life of a Volvox spheroid. If this synthesis is inhibited and/or this DNA species degraded in some manner, one would expect aberrant developmental patterns to emerge.

In this study Δ^9-THC has been shown to interfere specifically with cytoplasmic DNA metabolism, by both inhibiting its synthesis and causing its degradation. We are currently investigating the effects of administering this drug (as well as others which act on cytoplasmic DNA, e.g. Ethidium bromide) at specific points in the life cycle of synchronously grown Volvox spheroids. The morphological and biochemical results of these studies are giving us further understanding of molecular factors which influence development (Manuscript in preparation).

If the cytoplasmic specificity of the action of Δ^9-THC on the DNA of Volvox is shown to be a general phenomenon observable in other systems, then the anti-tumor effects of THC and its action as an inhibitor of DNA synthesis in in vitro mammalian systems may perhaps be due to its selective action on cytoplasmic (mitochondrial) DNA metabolism in these cell types. This important observation concerning the specificity of the molecular action of this controversial and widely used psychoactive agent on DNA should certainly be examined further.

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