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INFLUENCE OF Δ^9-TETRAHYDROCANNABINOL ON EXPRESSION OF HISTONE AND RIBOSOMAL GENES IN NORMAL AND TRANSFORMED HUMAN CELLS

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Abstract—The influence of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the cellular levels of histone mRNAs and ribosomal RNAs was examined in several normal and transformed human cell lines—HeLa S3 cells, WI-38 human diploid fibroblasts, SV40-transformed WI-38 cells, and A549 lung carcinoma cells. RNA sequences were quantitatively assayed by electrophoretic fractionation, transfer to nitrocellulose, and hybridization with cloned genomic human histone or ribosomal DNA sequences. Treatment with Δ^9-THC (10–40 μM) for 10 hr resulted in a concentration-dependent decrease in the representation of H2A, H2B, H3 and H4 histone mRNAs without a significant inhibitory effect on the levels of ribosomal RNAs. The cannabinoid-mediated inhibitory effect on histone gene expression was less evident in cells with active drug-metabolizing systems.

An assessment of the influence of cannabinoids on gene expression is central to defining the effects of these substances at the cellular and molecular levels. Several laboratories have reported concentration-dependent, cannabinoid-induced modifications in incorporation of radiolabeled precursors into RNA, suggesting that cannabinoids can influence transcription and/or the processing of RNA transcripts [1–20]. However, interpretation of these results is complicated by cannabinoid-induced fluctuations in radiolabeled precursor uptake and by inability to determine the influence of cannabinoids on specific gene transcripts.

In this paper we report the influence of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the representation of RNA transcripts from two defined genetic sequences, histone genes and ribosomal genes, in several human cell lines. Levels of cellular histone mRNAs and ribosomal RNAs were assayed by hybridization with cloned genomic human histone and ribosomal genes under conditions where quantitation was not influenced by nucleotide precursor pools. Our results suggest that Δ^9-THC causes a concentration-dependent inhibition in the cellular representation of histone mRNA sequences. This drug-induced inhibition is, at least to some extent, selective because cellular levels of ribosomal RNAs were not affected. Data are also presented which indicate that the cannabinoid-induced effect on histone gene expression is less pronounced in human cells with active drug-metabolizing systems.

MATERIALS AND METHODS

Cell culture. Exponentially growing (log phase) HeLa S3 cells, a hypotetraploid line of human cervical carcinoma cells, were maintained at 37° in suspension culture in Joklik-modified Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, NY) supplemented with 7% calf serum, 1.0 mM glutamine, 75 units/ml of potassium penicillin G, and 50 μg/ml of streptomycin sulfate [21].

WI-38 normal embryonic human diploid fibroblasts and SV40-transformed WI-38 human diploid fibroblasts were grown in monolayer culture in Basal Eagle's minimal essential medium supplemented with 10% fetal calf serum. A549 human lung carcinoma cells were grown in monolayer culture in Dulbecco's-modified Eagle's minimal essential medium supplemented with 10% calf serum [22, 23].

All experiments were carried out using exponentially growing cells, and all monolayer cultures were maintained in an incubator at 37° in a humidified 5% CO_2 atmosphere.

Drug administration. Delta^9-THC was supplied by the National Institute on Drug Abuse, and purity as determined by gas–liquid chromatography was 99%. The drug in 95% ethanol (as vehicle) was added directly to the suspension or monolayer cultures with a final ethanol concentration in all experiments adjusted to 0.15% (v/v). The "vehicle effect" was compensated by carrying out "vehicle only controls" and by treating cells with Δ^9-THC for 10 hr prior to monitoring biochemical and biological parameters. We observed that, after 1 hr of treatment with the drug vehicle (0.15% final concentration of ethanol), there was an inhibition (30–40%) in incorporation of [^{3}H]thymidine and [^{3}H]uridine into acid-insoluble material. This vehicle-mediated inhibition of radiolabeled precursor incorporation into nucleic acids was found to be reversible. When cells were treated with 0.15% ethanol for 3 or 6 hr prior to pulse labeling with [^{3}H]thymidine or [^{3}H]uridine, isotope incorporation into acid-insoluble material was also inhibited. Nine hours after introduction of the

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vehicle into the medium, isotope incorporation was indistinguishable from that occurring in untreated control, thus indicating the necessity for the 10-hr pretreatment. We have reported previously an extensive description of biochemical parameters associated with treatment of human tissue culture cells with ethanol [12, 13].

While it is difficult to compare drug levels in cell cultures with those in intact organisms, studies from a number of laboratories indicate that, in most mammals studied, the blood concentrations which are produced by minimum effective to moderately high doses of Δ^9-THC (7–10 ml/kg) are between 10^{-8} and 10^{-4} M [24, 25].

Cell viability determinations. As reported previously, cell viability, as determined by trypan blue exclusion, was greater than 98% for control, vehicle-treated, and cannabinoid-treated cultures [12, 13].

Preparation of total cellular RNA. Cells (5×10^7) were lysed in 3.0 ml of a solution containing 1.6 mM Tris–HCl (pH 7.4), 0.8 mM EDTA, 5.0 μg/ml polyvinylsulfonic acid (PVS), 2.0% (w/v) sodium dodecyl sulfate (SDS) and 0.5 mg/ml proteinase K. After a 15-min incubation at room temperature, and the addition of 0.15 ml of a 5 M NaCl solution, the aqueous phase was extracted once with 1 vol. of buffered phenol–chloroform–isoamyl alcohol (25:24:1, by vol.), and twice with 1 vol. of chloroform–isoamyl alcohol (24:1, v/v). Nucleic acids were precipitated with 3 vol. of ethanol at −20° in the presence of 0.4 M sodium acetate.

Nucleic acids were recovered by centrifugation, resuspended in 2 ml of 10 mM Tris–HCl (pH 7.5), 2 mM $CaCl_2$, 10 mM $MgCl_2$, and 5.0 μg/ml PVS, and incubated at 37° for 20 min in the presence of 0.1 mg/ml DNase I (Sigma electrophoretically pure) which had been pretreated with proteinase K for 2 hr as described by Tullis and Rubin [21]. After addition of 0.25 vol. of 10% (w/v) SDS, the RNA solution was extracted with phenol and chloroform, and ethanol precipitated as described above.

Nitrocellulose blot analysis of total cellular RNA. Total cellular RNA (50 μg determined spectrophotometrically) was resolved electrophoretically in 1.5% (w/v) agarose gels containing 6% (w/v) formaldehyde as described elsewhere [26], except that a mix of 6% (w/v) formaldehyde, 50 mM boric acid, 5 mM sodium borate, 10 mM sodium sulfate, and 1 mM EDTA (pH 8.19) was used as the electrolyte. RNA was transferred to nitrocellulose in 20x SSC [3 M NaCl, 0.3 M sodium citrate (pH 7.25)] as recommended by Southern [27], and the filters were then baked *in vacuo* for 2 hr at 80°.

Filters were prehybridized at 45–50° for 4 hr in hybridization buffer–50% (w/v) formamide, 5x SSC, 250 μg/ml *Escherichia coli* DNA, and 5x Denhardt's solution [10x Denhart's is 0.2% (w/v) Ficoll, 0.2% (w/v) polyvinylpyrrolidone]. Hybridization was performed at 45–50° for 21–24 hr in hybridization buffer containing 10^6 cpm/ml of thermally denatured probe. The ^{32}P-labeled DNA probe was prepared by nick-translation with [^{32}P]dCTP as described by Maniatis *et al.* [28].

Prior to autoradiography, filters were washed once at room temperature for 10 min with 100 ml of 5x SSC, 1x Denhardt's, and then at 65° for 30 min with 25 ml each of the following: (i) 5x SSC, 1x Denhardt's; (ii) 2x SSC, 0.1% (w/v) SDS; (iii) 1x SSC, 0.1% (w/v) SDS; and (iv) 0.1x SSC, 0.1% (w/v) SDS. Filters were exposed to preflashed Kodak XAR 5 or Chronex X-ray film at −70° for 20–40 hr in cassettes containing Kodak regular intensifying screens.

Purification of cloned human histone gene sequences. The isolation and characterization of λCh 4A recombinants containing human histone genes have been described [29]. EcoRI-EcoRI or EcoRI-HindIII restriction fragments of the recombinant DNAs have been subcloned into the plasmid pBR322, transformed into *E. coli* strain HB101, and characterized further by hybrid selection—*in vitro* translation and partial sequencing. All recombinant DNA manipulations were carried out in accordance with the guidelines established by the National Institutes of Health.

Plasmid DNA was isolated by the sarcosyl lysis procedure and purified further by CsCl buoyant density gradient centrifugation essentially as described by Clewell and Helinski [30].

RESULTS

Influence of Δ^9-THC on cellular levels of human histone mRNAs in HeLa S3 cells. Initially, the steady-state levels of histone mRNAs were determined in exponentially growing human cervical carcinoma cells, HeLa S3 cells, after treatment with increasing concentrations of Δ^9-THC. Total cellular RNAs were isolated from untreated control cells, vehicle-treated control cells, and cells treated with 10, 30, or 40 μM concentrations of Δ^9-THC. These RNAs were fractionated electrophoretically in 1.5% agarose gels [26], transferred to nitrocellulose [27], and hybridized with ^{32}P-labeled cloned genomic human histone sequences [29]. The levels of histone mRNAs were then assayed autoradiographically.

Isolation of total cellular RNA permits greater than 90% recovery, circumventing loss of RNA through nuclease activity and physical manipulations which generally occur during subcellular fractionation. Because the hybridization probe is radiolabeled *in vitro*, rather than the cellular RNAs *in vivo*, quantitation of RNAs is not complicated by the intracellular ribonucleotide precursor pools. RNA samples are quantitated spectrophotometrically prior to electrophoretic fractionation, and the extent of transfer to nitrocellulose is monitored by ethidium bromide staining and/or ultraviolet shadowing prior to and following diffusion transfer. The efficiency of transfer to nitrocellulose by the procedure utilized in these experiments has been monitored by transfer of ^{32}P-labeled DNA and shown to be greater than 95%.

The data in Figs. 1 and 2 clearly indicate that Δ^9-THC brings about a concentration-dependent decrease in the representation of mRNAs for the four core histone proteins, H2A, H2B, H3 and H4. Shown in Fig. 1A are the hybridization signals obtained when 50 μg of nitrocellulose-immobilized, total cellular HeLa cell RNAs from control and Δ^9-THC-treated cells were hybridized with a cloned

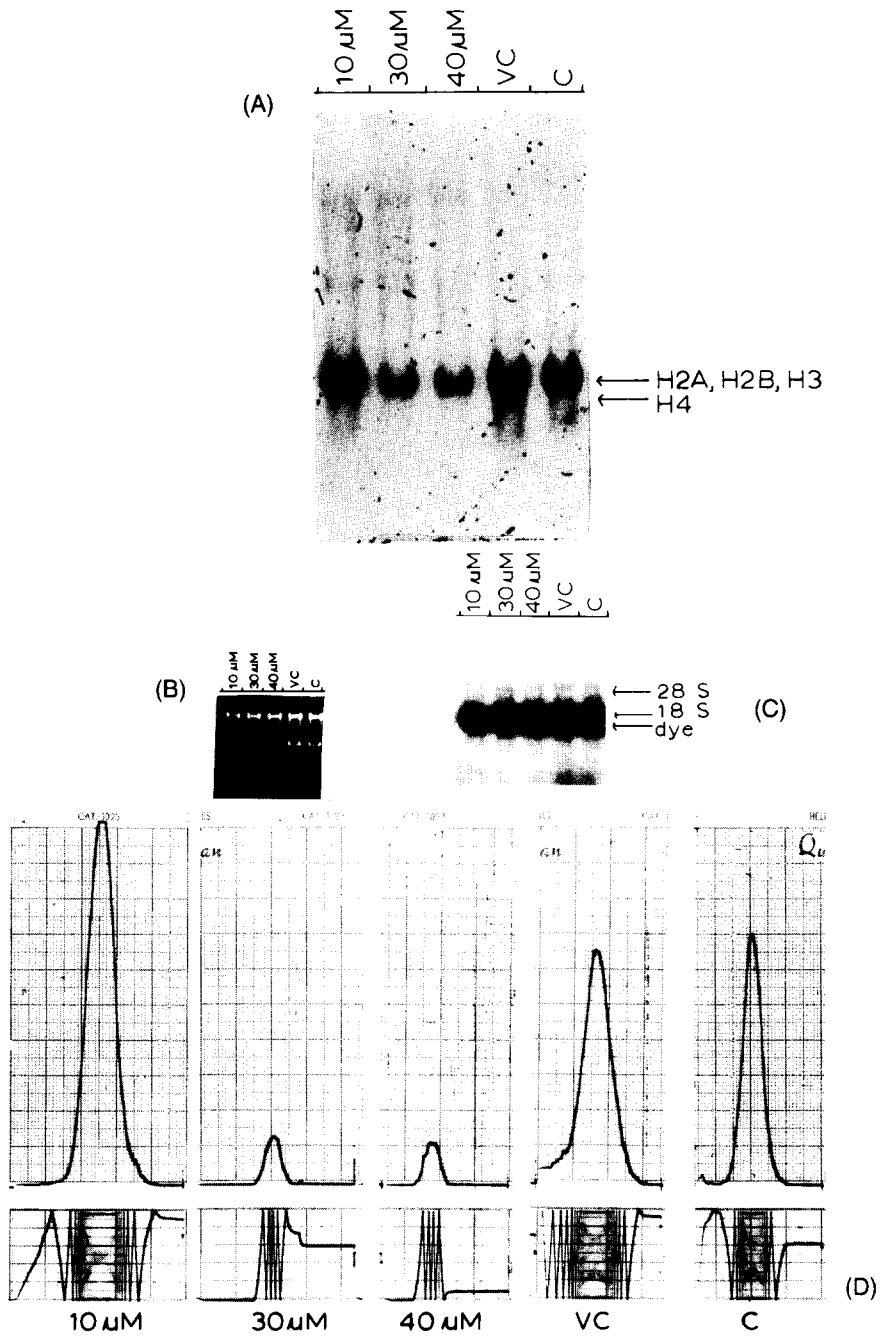

Fig. 1. (A) Effects of various concentrations (10, 30 and 40 μM; VC, vehicle-treated control and C, control) of Δ^9-THC on the representation of mRNAs for three of the four core histone proteins, H2A, H2B and H3. The signals shown were obtained when 50 μg of electrophoretically fractionated nitrocellulose-immobilized total cellular HeLa cell RNAs were hybridized to a cloned human DNA sequence (pFF435) encoding H2A, H2B and H3 histone mRNAs. (B) Ethidium bromide stain of 1.5% (w/v) agarose gel with 6% (w/v) formaldehyde [26], containing 10 μg of each of the Δ^9-THC-treated and control samples of total cellular RNAs from HeLa cells. The gel was stained for 1 hr in 0.1 M ammonium acetate containing 0.1 μg/ml ethidium bromide and destained overnight in water. The gel was placed on a shortwave ultraviolet transilluminator and photographed with polaroid type 57 film using an orange filter. (C) Ultraviolet shadowing of 1.5% (w/v) agarose gel with 6% (w/v) formaldehyde [26], containing 50 μg of each of the Δ^9-THC-treated and control samples of total cellular RNAs from HeLa cells. The gel was placed on a cellulose-fluorescent thin-layer chromatography plate and illuminated from above by shortwave ultraviolet light. The gel was photographed with polaroid type 57 film using an orange filter. (D) Densitometric scan of autoradiographic hybridization signals obtained when 50 μg of electrophoretically fractionated nitrocellulose-immobilized total cellular RNAs from HeLa cells treated with various concentrations of Δ^9-THC were hybridized to a cloned human DNA sequence (pFF435) encoding H2A, H2B and H3 histones. The top portion of the scan measures the absorbance of the signal which is determined electronically within the densitometer based on the measured optical density. The lower portion is the Zig–Zag time base integrator and is used to quantitate the area under the curve and, thus, the concentration of the sample.

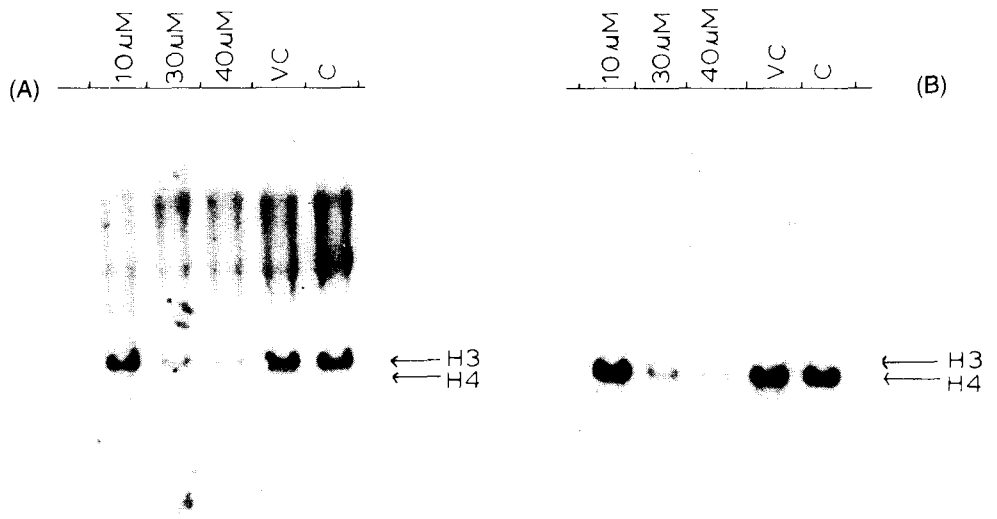

Fig. 2. Effects of various concentrations (10, 30 and 40 μM; VC, vehicle-treated control and C, control) of Δ^9-THC on the representation of mRNAs for histones H3 and H4. The signals shown were obtained when 50 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular HeLa cell RNAs were hybridized to cloned human DNA sequences encoding: (A) H3 histone (pF0422) and (B) H4 histone (pF0108A).

human DNA sequence (pFF435) encoding H2A, H2B and H3 histone mRNAs. While the levels of H2A, H2B and H3 histone mRNAs isolated from cells treated with 10 μM Δ^9-THC were not below those from non-drug-treated or vehicle-treated controls, a marked inhibition (greater than 80%—see Table 1) was observed in cells treated with 30 and 40 μM drug concentrations. Verification that equivalent amounts of all RNA samples were fractionated can be gleaned from Fig. 1B which shows similar levels of ethidium bromide staining of all RNAs and from Fig. 1C which shows similar levels of all RNAs by ultraviolet shadowing. It should be noted that, because equivalent amounts of RNA from control and drug-treated cells were analyzed, the data in Fig. 1A reflect a concentration-dependent, Δ^9-THC-mediated inhibition in the relative representation of three core histone mRNA species. A concentration-dependent inhibition of the absolute amounts of H2A, H2B and H3 histone mRNA/cell, with pronounced inhibition evident at 30 and 40 μM drug concentrations, was also observed when equivalent aliquots (by volume) of RNA extracts from equivalent numbers of control and Δ^9-THC-treated cells were similarly analyzed (see Fig. 1D). The data in Fig. 2 are results from experiments in which total cellular RNAs from control and Δ^9-THC-treated, exponentially growing HeLa S3 cells were analyzed by hybridization with cloned genomic H3 (pF0422) (Fig. 2A) or H4 (pF0108A) (Fig. 2B) histone sequences. Consistent with the results shown in Fig. 1, a greater than 80% inhibition in the representation of H3 and H4 histone mRNAs was observed following treatment with 30 and 40 μM drug concentrations.

Influence of Δ^9-THC on cellular levels of histone mRNAs in WI-38 human diploid fibroblasts and in SV40-transformed WI-38 cells. The influence of Δ^9-THC on the levels of histone mRNAs was then studied in normal diploid cells (WI-38 human diploid fibroblasts) and in SV40-transformed WI-38 cells. A concentration-dependent, drug-induced decrease in the levels of all four core histone mRNAs was observed in both normal human diploid fibroblasts and in SV40-transformed human diploid fibroblasts—a cannabinoid-induced inhibition similar to that seen in HeLa S3 cells. As shown in panels A and B of Fig. 3, when total cellular RNAs from control and Δ^9-THC-treated WI-38 cells were hybridized with ^{32}P-labeled pFF435, a plasmid containing cloned

Table 1. Effect of Δ^9-THC on cellular levels of human (HeLa) histone mRNAs*

Treatment	Drug concn	% Inhibition
Δ^9-THC	10 μM	0
Δ^9-THC	30 μM	78.1
Δ^9-THC	40 μM	81.0
Vehicle control	0	0
Control	0	0

* Cells were treated with the indicated concentrations of Δ^9-THC. RNAs were isolated, fractionated electrophoretically, transferred to nitrocellulose, and hybridized with ^{32}P-labeled cloned genomic human histone sequences. These techniques have been described in Materials and Methods. Following autoradiography, histone mRNA levels were quantitated by densitometry.

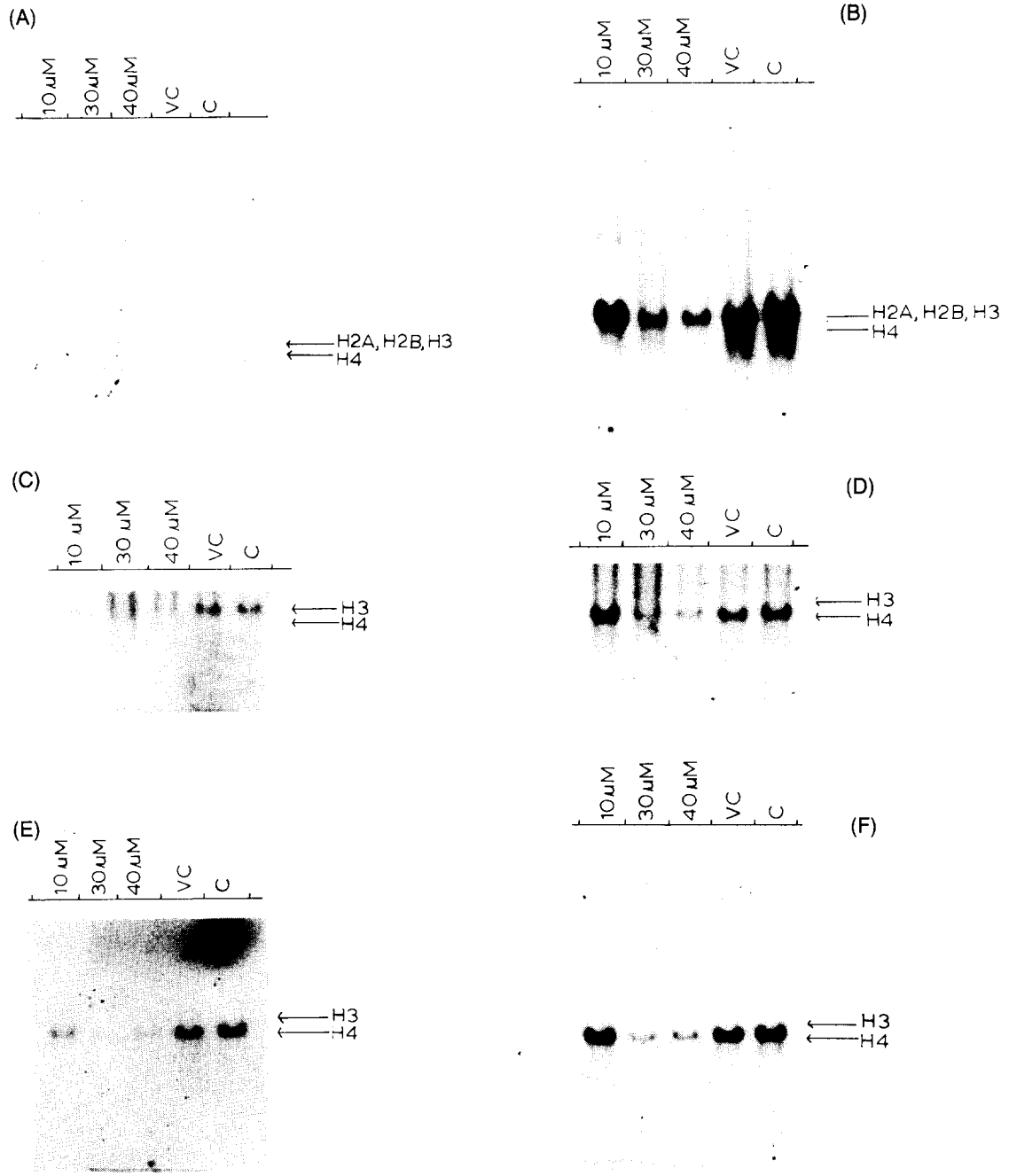

Fig. 3. Effects of various concentrations (10, 30 and 40 μM; VC, vehicle-treated control and C, control) of Δ⁹-THC on the representation of mRNAs for the four core histones in normal, diploid, and transformed human cells. The signals shown were obtained when 50 μg of electrophoretically fractionated nitrocellulose-immobilized total cellular RNAs were hybridized to cloned human DNA sequences. (A) WI-38 and (B) SV40-WI-38 total cellular RNA hybridized to a DNA probe (pFF435) encoding H2A, H2B and H3 histones; (C) WI-38 and (D) SV40-WI-38 total cellular RNA hybridized to a DNA probe (pF0422) encoding H3 histone; (E) WI-38 and (F) SV40-WI-38 total cellular RNA hybridized to a DNA probe (pF0108A) encoding H4 histone.

human genomic H2A, H2B and H3 histone coding sequences, decreased levels of histone mRNAs were observed in both normal WI-38 and in SV40-transformed WI-38 cells treated with 30 and 40 μM drug concentrations. Confirmation of the Δ⁹-THC-mediated inhibition of core histone mRNA levels in normal and SV40-transformed WI-38 human diploid fibroblasts can be seen in panels C and D as well as in panels E and F where similar drug-induced inhibitions in the representation of H3 and H4 mRNAs, respectively, were observed.

Influence of Δ⁹-THC on cellular levels of histone mRNAs in A549 human lung carcinoma cells. The levels of H2A, H2B, H3 and H4 histone mRNAs

Table 2. Cellular uptake and subcellular distribution of [^{3}H]Δ^9-THC*

Cell type	cpm/10^7 cells	% Nucleus	% Cytoplasm
SV40-WI-38	1.2×10^5	32.6	67.4
A549	1.3×10^5	37.9	62.1

* Cells (4×10^7) were treated with 30 μM Δ^9-THC (10 μCi/mg of [^{3}H]Δ^9-THC, obtained from the National Institute on Drug Abuse) for 30 min. Half of the cells were solubilized in protosol. The remaining cells were lysed in 80 mM NaCl, 20 mM EDTA, 1% Triton X-100, pH 7.2, and the nuclear and cytoplasmic fractions were separated by centrifugation. The nuclei were solubilized in protosol. Aliquots (100 μl) were taken from each fraction (total cells, nuclei and cytoplasm) and counted in Triton–toluene scintillation fluid using a Beckman LS-230 liquid scintillation counter. Cellular uptake of Δ^9-THC ranged between 3.5 and 3.9%.

were similarly assayed in A549 human lung carcinoma cells after treatment with Δ^9-THC. These cells have been reported to have active drug-metabolizing systems and to efficiently metabolize polycyclic hydrocarbon-containing carcinogens [23]. A pronounced decrease in the inhibitor effect of Δ^9-THC on the representation of core histone mRNAs was observed in A549 cells compared with HeLa S3 cells and WI-38 cells (normal and SV40-transformed). It is unlikely that the reduced sensitivity of A549 cells to cannabinoid treatment was attributable to changes in drug uptake. The intracellular levels of Δ^9-THC in SV40-transformed WI-38 cells and in A549 cells, when monitored by intracellular incorporation of [^{3}H]Δ^9-THC (Table 2), did not reflect the differences seen in histone mRNA levels (Figs. 3 and 4).

Influence of Δ^9-THC on cellular levels of ribosomal RNAs. In all experiments reported in this paper, the representation of 18S and 28S ribosomal RNAs was monitored in control and in Δ^9-THC-treated cells by staining gels with ethidium bromide and by ultraviolet shadowing. A typical example of a gel showing the levels of the major ribosomal RNAs in control and in drug-treated cells is shown in panels B and C of Fig. 1. Additionally, when electrophoretically fractionated cellular RNAs from control and Δ^9-THC-treated cells were hybridized with ^{32}P-labeled cloned human 18S (LS-2) and 28S (LS-6) ribosomal RNA coding sequences, a dose-dependent decrease in the representation of these RNAs was not observed. Panels A and B of Fig. 5 show no changes in the levels of 28S ribosomal RNAs from Δ^9-THC-treated HeLa and SV40-transformed WI-38 cells in the same RNA samples where greater than 80% reduction was observed for the representation of core histone mRNAs in treated cells. Panel C of Fig.

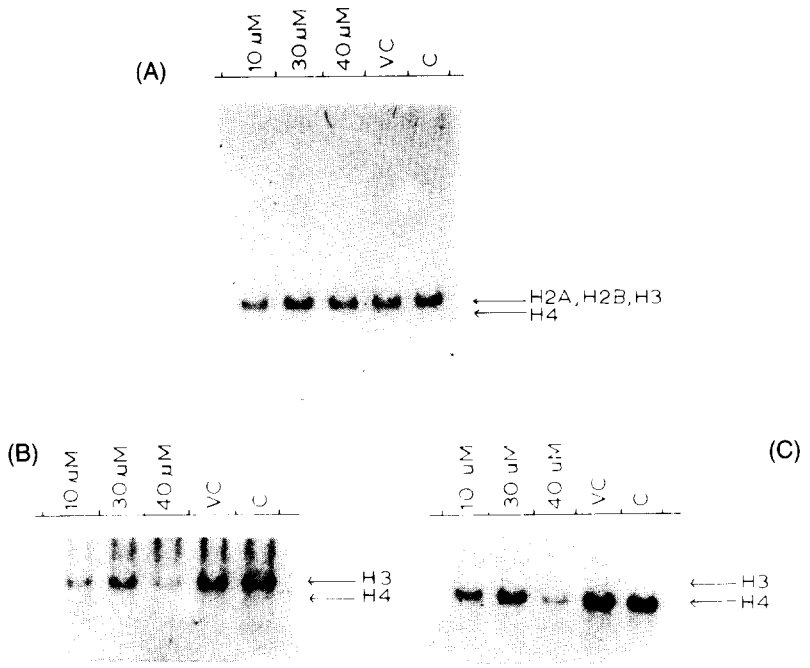

Fig. 4. Effects of various concentrations (10, 30 and 40 μM; VC, vehicle-treated control and C, control) of Δ^9-THC on the representation of mRNAs for the four core histones in A549 human tumor cells. The signals shown were obtained when 50 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular RNAs from A549 cells were hybridized to cloned human DNA sequences coding for: (A) H2A, H2B and H3 histone (pFF435); (B) H3 histone (pF0422); and (C) H4 histone (pF0108A).

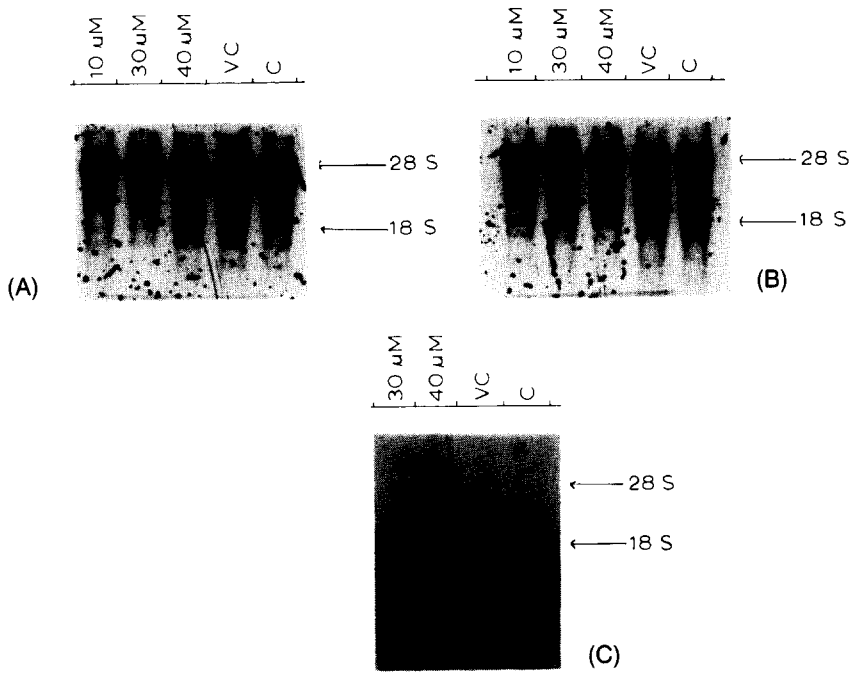

Fig. 5. Effects of various concentrations (10, 30 and 40 μM; VC, vehicle-treated control and C, control) of Δ^9-THC on the representation of 28S and 18S ribosomal RNAs. The signals shown were obtained with 10 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular RNAs were hybridized to cloned human DNA sequences. (A) HeLa and (B) SV40-WI-38 total cellular RNA hybridized to a DNA probe (LS-6) encoding 28S RNA; (C) HeLa total cellular RNA hybridized to a DNA probe (LS-2) encoding 18S RNA.

5 shows unchanged levels of 18S ribosomal RNA in these same cells following hybridization with ^{32}P-labeled human 18S ribosomal DNA.

DISCUSSION

We examined the influence of Δ^9-THC on the expression of human histone and ribosomal genes. These genetic sequences represent two distinct types of human genes which differ with respect to their organization, regulation and functions. Human histone genes are a family of moderately reiterated genetic sequences—approximately 40 copies/haploid genome. Each histone mRNA is transcribed from a set of contiguous nucleotide sequences (unspliced), and histone gene expression is related to cell proliferation. The gene products, the histone proteins, are required for packaging several yards of DNA into "nucleosomes" where they are contained in a nucleus only several microns in diameter. The histone proteins are necessary for genome replication (to package newly replicated DNA) and additionally play a role in the control of gene expression. The human ribosomal genes are also represented as a reiterated set of sequences (200 copies/haploid genome), and the final gene products are the major structural RNA species associated with large and small ribosomal subunits. In contrast to the histone genes, where the primary transcripts undergo a minimal amount of processing, the 5.8S, 18S and 28S ribosomal RNAs are derived from a 45S precursor via a series of post-transcriptional cleavages.

A long-standing question has been whether cannabinoids influence the expression of specific genetic sequences. While cannabinoid-induced effects on cell structure and function, coupled with cannabinoid-mediated modifications in macromolecular biosynthesis, are consistent with such a contention, direct experimental evidence for an effect of cannabinoids on expression of specific genes has, to date, not been reported. In this paper we present data which indicate that treatment of exponentially growing normal diploid and transformed human cells with Δ^9-THC resulted in a concentration-dependent decrease in the representation of histone gene transcripts, with a decreased sensitivity of cells with highly developed drug-metabolizing systems.

Several lines of experimental evidence suggest that the Δ^9-THC-induced reductions in histone mRNA levels that we have observed in normal and transformed human cells are not merely a reflection of a general, non-specific, cannabinoid-induced inhibition in RNA synthesis. As reported previously, the cannabinoid-induced inhibition of [^{3}H]uridine incorporation into total cellular RNAs largely reflects a drug-induced influence on the intracellular nucleotide precursor pool rather than an effect on cellular RNA metabolism [12, 13]. The absence of a significant quantitative effect of psychoactive and nonpsychoactive cannabinoids on levels of nuclear [12, 13] or chromatin [12, 13] transcription *in vitro* further suggests that these drugs do not interfere with the general levels or rates of cellular RNA synthesis.

The inability of Δ^9-THC, at concentrations be-

tween 10 and 40 μM, to modify the levels of ribosomal RNAs provides more direct evidence for some extent of specificity to the cannabinoid-mediated decrease in histone mRNA levels.

While our results clearly indicate that Δ^9-THC preferentially inhibits expression of histone genes, the levels at which regulation are perturbed and the biological implications of this cannabinoid-mediated effect remain to be resolved. The reduction in cellular levels of histone mRNAs after cannabinoid treatment may be attributable to alterations in mRNA stability, transcription, or processing of histone transcripts. Alternatively and/or additionally, drug-induced structural modifications in the histone genes and in their flanking regulatory sequences should also be considered within this context. By analogy with other moderately reiterated eukaryotic sequences which have been shown to undergo structural modifications in conjunction with phenotypic changes, cannabinoid-induced effects on the structural features of human histone genes could be a viable possibility. The extent to which expression of specific genetic sequences other than histone sequences is affected by cannabinoids is also an open-ended question—one which is particularly important because the organization and regulation of the moderately reiterated human histone genes differ considerably from those of the more complex, spliced single copy genes.

From a biological standpoint the selective effect of Δ^9-THC on expression of histone genes may be understandable. Expression of histone genes has been shown to be temporally and functionally coupled with DNA replication [21, 31–36], and cannabinoids have been shown to bring about a concentration-dependent inhibition in cell proliferation [1–20]. In fact, in the normal and transformed cell lines that we have examined, the extent to which histone mRNA levels are affected by Δ^9-THC is paralleled by the extent to which proliferative activity is affected by cannabinoids. It remains to be determined whether expression of other genetic sequences, whose expression is prerequisite for DNA replication or mitotic division, is preferentially inhibited by cannabinoids. Equally important is whether the cannabinoid-mediated modifications in cellular histone mRNA levels are attributable to a direct effect on the histone genes and/or the transcripts, or alternatively, whether the effects of cannabinoids on histone gene expression are indirect, e.g. acting initially on other genetic sequences or cellular macromolecules.

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