PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **185**, 41–48 (1987)

Inhibition of Cell-Associated Herpes Simplex Virus Type 2 Glycoproteins by Δ^9-Tetrahydrocannabinol (42514)

ERIC M. MISHKIN AND GUY A. CABRAL[1]

Department of Microbiology and Immunology, Virginia Commonwealth University, Box 678, MCV Station, Richmond, Virginia 23298-0001

Abstract. This study was conducted to define the effect of micromolar concentrations of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the biosynthesis and expression of herpes simplex virus type 2 (HSV2)-specified glycoproteins. Dose-related reductions in all species of virus glycoproteins were recorded by one-dimensional SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and autoradiography of [^{14}C]glucosamine-labeled infected Vero cells treated with 10^{-7} to 10^{-5} M Δ^9-THC. A drug dose-related depletion of the mature HSV2 major envelope glycoprotein complex (119-kDa average molecular weight), accompanied by accumulation of immature unglycosylated species, was demonstrated by two-dimensional SDS–PAGE in concert with Western immunoblotting or autoradiography. Light and electron microscopy immunoperoxidase staining revealed that Δ^9-THC effected depletion of 119-kDa determinants from the infected cell surface. This depletion occurred concomitantly with accumulation of 119-kDa components at the perinucleus. However, the expression of 119-kDa glycoproteins on the virion envelope was not affected. These results indicate that Δ^9-THC inhibits the synthesis, maturation, and cellular transport of HSV2-specified glycoproteins. Decreased expression of virus glycoproteins on the infected cell surface may affect host immune responsiveness to HSV2. © 1987 Society for Experimental Biology and Medicine.

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of marihuana, has been shown to exert a spectrum of effects both *in vivo* and *in vitro*. The drug affects immunoresponsiveness both in humans (1) and animals (2) and decreases host resistance to bacterial and viral pathogens (3). Δ^9-THC also inhibits macromolecular synthesis in a diverse number of cell culture systems (4–6). This laboratory has previously reported that Δ^9-THC at micromolar levels inhibits the synthesis of herpes simplex virus type 2 (HSV2) cell-associated structural proteins (7). The drug doses which effected this macromolecular inhibition, however, did not significantly alter the total production of infectious HSV2. These observations suggested that Δ^9-THC differentially inhibited the expression of HSV2-specified, cell-associated proteins. Since cell-associated virus gene products comprise the bulk of viral antigen presented to immunocytes in the infected host (8), drug-induced suppression of their expression, especially of glycoproteins, could modify immune recognition and subsequent induction of cellular and humoral immunity. Thus, the purpose of this investigation was to define the *in vitro* effect of micromolar concentrations of Δ^9-THC on the cell-associated biosynthesis and expression of the major glycoprotein classes of HSV2.

Materials and Methods. *Cells.* Green monkey kidney (Vero) cells were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum (FCS) and penicillin (20 U/ml)/streptomycin (20 μg/ml)/ Fungizone (0.05 μg/ml) (GIBCO, Grand Isle, NY). Cells were maintained in the same medium supplemented with 5% FCS.

Drugs. Δ^9-THC was supplied by the National Institute of Drug Abuse (NIDA, Rockville, MD). Drug concentrations of 10^{-5}, 10^{-6}, and 10^{-7} M were prepared by dissolving 10 μl of appropriately diluted (in 95% ethanol) stock cannabinoid per milliliter of culture medium. The vehicle consisted of medium containing 10 μl of 95% ethanol/per milliliter. Thus, medium containing Δ^9-THC or vehicle was at a 1% ethanol final concentration.

Radiolabel. D-[^{14}C(U)]Glucosamine (sp act 328.5 mCi/mM) was purchased from New England Nuclear (Boston, MA).

Virus. HSV2, strain P180/HSV2/ST (FMC), was isolated from a gynecological pa-

[1] To whom correspondence should be addressed.

0037-9727/87 $1.50
Copyright © 1987 by the Society for Experimental Biology and Medicine.
All rights reserved.

tient and was typed by partial *Eco*RI restriction endonuclease digestion (9). Virus stocks were shown by plaque assay (10) on Vero cell monolayers to contain 1×10^9 PFU/ml.

Drug administration and virus infection. Preliminary studies employing different dosing regimens relative to time of virus inoculation indicated no significant qualitative differences on virus infection. Subsequent studies employed 24-hr preincubation of cell monolayers with drug, vehicle, or placebo (i.e., medium) at 37°C (5% CO_2). Cells, then, were washed with sterile PBS and were inoculated with HSV2 at a multiplicity of infection (MOI) of 5 PFU/cell. Virus was allowed to absorb for 1 hr (37°C, 5% CO_2) and maintenance medium, then, was added. In studies involving radiolabeling, D-[^{14}C(U)]glucosamine (25 μCi/ml) was added at 4-hr postviral inoculation (pi).

SDS–polyacrylamide gel electrophoresis (SDS–PAGE). Cultures were harvested at 24 hr pi and were processed for either one- or two-dimensional SDS–PAGE. For one-dimensional SDS–PAGE, equal volumes (i.e., 25 μl) of aqueous cell homogenate and solubilization buffer were mixed to yield a final concentration of 2% SDS, 0.5% β-mercaptoethanol, and 10% glycerol in Tris–HCl buffer (pH 6.8). Bromophenol blue was added to samples immediately before electrophoresis in 10% polyacrylamide gel slabs at 15 mA/gel using the discontinuous buffer system of Ornstein (11) and Davis (12). Following electrophoresis, gels containing the separated radiolabeled virus-specified glycoproteins were stained with Coomassie brilliant blue, destained, vacuum dried, and processed for radioautography by exposure of XRP-5 X-ray film (Kodak, Rochester, NY). Protein profiles were quantitated by densitometry (A-E Apparatus Co., St. Petersburg, FL). Two-dimensional SDS–PAGE was performed after the method of O'Farrell (13). Cultures, harvested in 0.2 ml of lysis buffer, were subjected to isoelectric focusing in a pH gradient ranging from 4.5 to 7.8 in the first dimension. The second dimension consisted of 10% SDS–PAGE run at 20 mA/gel. Resultant gels were processed as described above.

Western immunoblotting. Western immunoblotting was performed after the method of Symington (14). Briefly, virus-specified proteins separated by one- or two-dimensional SDS–PAGE were transferred to nitrocellulose membrane at 90 mA for 18 hr. The nitrocellulose membrane, then, was reacted with a 1:350 dilution of rabbit anti-serum specific for the 119-kDa (i.e., 119,000 Da) HSV2 major envelope glycoprotein complex. The anti-119-kDa antiserum was prepared by immunization of rabbits with the corresponding purified HSV-specified protein complex obtained by sequential polypreparative and cylindrical SDS–PAGE as previously described (15, 16). The anti-119-kDa antiserum was absorbed with liver powder and 10^8 Vero cells/ml prior to use. The secondary antibody consisted of peroxidase-conjugated goat IgG anti-rabbit IgG heavy and light chains (1:2000; Cappel, Malvern, PA). Nitrocellulose membranes were developed using HRP color development solution (Bio-Rad, Richmond, CA).

Light microscopy immunoperoxidase staining. For surface staining of 119-kDa determinants by immunoperoxidase, subconfluent drug-treated, virus-infected cells, grown on coverslips, were fixed in 4% formaldehyde in phosphate-buffered saline (PBS). The anti-119-kDa antiserum and the horseradish peroxidase-conjugated secondary antibody were the same as described for immunoblotting and were employed at 1:3 and 1:10 dilutions, respectively. Cultures were reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB, 0.5 mg/ml) in Tris buffer (pH 7.6) containing 0.01% H_2O_2. Coverslips were dehydrated, mounted in Permount (Fisher Scientific, Richmond, VA), and viewed in a BH2 Olympus microscope (Opelco, Washington, DC). Internal immunoperoxidase staining was performed as described above except that cells were fixed with absolute acetone at room temperature for 10 min prior to staining.

Electron microscopy immunoperoxidase staining. For surface immunoperoxidase staining, drug-treated virus-infected cells were prefixed in 0.025 *M* Sorensen's phosphate buffer (SPB, pH 7.2) containing 0.25 *M* sucrose, 2% paraformaldehyde, and 0.05% glutaraldehyde for 20 min. Cultures were processed as described for light microscopy, except that following development for immunoperoxidase staining, cells were postfixed in 2% glutaraldehyde in SPB, processed for transmission electron microscopy (17), and viewed in a Zeiss EM10-CA (Carl Zeiss, New York,

NY) operating at an accelerating voltage of 60 kV.

Results. *Effect of Δ^9-THC on the biosynthesis of HSV2-specified glycoproteins.* Densitometry of profiles of [^{14}C]glucosamine-labeled cultures pretreated for 24 hr with Δ^9-THC revealed 32 to 50% reductions in all mature species of virus-specified glycoproteins (Fig. 1). These reductions were effected for drug doses ranging from 10^{-7} to 10^{-5} *M*. Parallel cultures processed by plaque assay showed no significant differences in the production of total infectious virus in agreement with previous studies (18). Representative alterations in HSV2-glycoprotein biosynthesis were obtained at 10^{-7} *M* Δ^9-THC where 44, 49, and 47% decreases in peak absorbance in the 133-, 111-, and 105-kDa glycoproteins corresponding to gC, gB, and pgB (i.e., gA), respectively, occurred when compared with vehicle-treated, virus-infected controls. These glycoprotein species are constituent subpopulations of the 119-kDa average molecular weight HSV2 major envelope glycoprotein complex. Depletion (35%) of the 92-kDa species, corresponding to gE (19, 20), was recorded at 10^{-7} *M* Δ^9-THC. In addition, decreases in the 65-kDa (31%), 54-kDa (45%), 51-kDa (68%), and 42-kDa (37%) species were noted. The 54- and/or 51-kDa species may correspond to glycoprotein gD (20, 21).

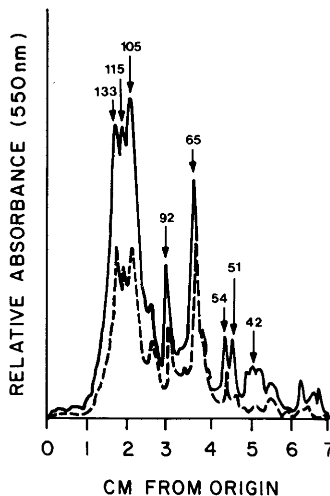

FIG. 1. Densitometry of SDS–polyacrylamide gel electrophoresis profiles of [^{14}C]glucosamine-labeled HSV2-infected Vero cell monolayers. The numbers correspond to the average molecular weight of each species in kilodaltons. The solid line represents the profile for HSV2-specified cell-associated glycoproteins from cells pretreated for 24 hr with vehicle. The dashed line represents the HSV2-specified cell-associated glycoprotein profile for cells pretreated for 24 hr with 10^{-7} *M* Δ^9-THC. Reductions ranging from 32 to 50% in all mature glycoprotein species were observed.

Effect of Δ^9-THC on glycoprotein processing. To further define the effect of Δ^9-THC on HSV2-glycoprotein biosynthesis, two-dimensional SDS–PAGE of [^{14}C]glucosamine-labeled virus-infected cell homogenates and two-dimensional SDS–PAGE in concert with Western immunoblotting were performed. Western immunoblotting employing anti-119 kDa antiserum demonstrated moderate drug dose-related reductions of transferred protein when compared to vehicle controls. However, cannabinoid-related alterations in the isoelectric mobility of components of the major envelope glycoprotein complex were noted (Fig. 2). These alterations were characterized as a dose-related depletion of the more acidic species of the 119-kDa complex coupled with accumulation of relatively less acidic species demonstrating minor reductions in apparent molecular weight. Autoradiography of two-dimensional gels of [^{14}C]glucosamine-labeled HSV2 glycoproteins revealed overt drug dose-related reductions in the incorporation of glucosamine derivatives in all species of HSV2-specified glycoproteins (Fig. 3). These reductions in [^{14}C]glucosamine incorporation into 119-kDa components were comparably greater than those noted for total 119-kDa transferred protein observed by Western immunoblotting.

Effect of Δ^9-THC on the localization of HSV2-specified glycoproteins. To determine the effect of Δ^9-THC on the expression of the 119-kDa virus-specified glycoprotein complex on the virus envelope as well as on, and within, virus-infected cells, light and electron microscopy immunoperoxidase studies were conducted (Fig. 4). Light microscopy of surface immunoperoxidase staining revealed qualitative drug dose-related reductions in the expression of cell surface-associated 119-kDa determinants. At 10^{-5} *M* Δ^9-THC little staining was observed on the surface of virus-infected cells. Immunoperoxidase light microscopy staining for the intracellular 119-kDa glycoprotein complex in virus-infected, vehi-

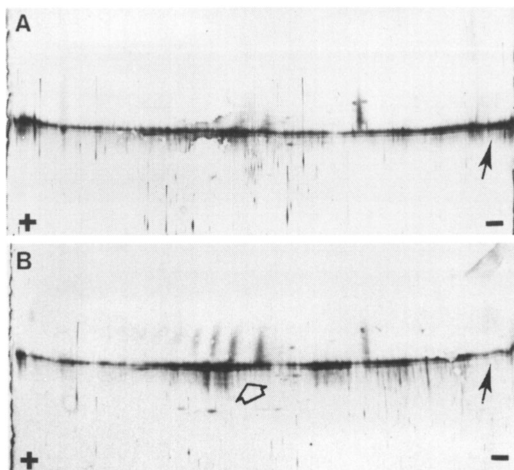

FIG. 2. Two-dimensional SDS–polyacrylamide gel analysis of HSV2-specified cell-associated 119-kDa glycoprotein expression in cells pretreated with vehicle or with Δ^9-THC for 24 hr. Solubilized whole cell homogenates were subjected to isoelectric focusing in a pH gradient ranging from 4.5 to 7.8 in the first dimension and 10% SDS–polyacrylamide gel electrophoresis in the second dimension. Separated proteins, then, were electrophoretically transferred to nitrocellulose membrane and processed for the demonstration of 119-kDa determinants by immunoperoxidase staining as described under Materials and Methods. (A) Immunostaining for 119-kDa determinants in homogenates of cells pretreated with vehicle. (B) Immunostaining pattern for cells pretreated with 10^{-5} *M* Δ^9-THC. Note the depletion of the more acidic species of the 119-kDa complex (solid arrow) coupled with accumulation of the relatively less acidic species (open arrow) when compared to vehicle controls.

cle-treated cells demonstrated a normal distribution of 119-kDa components dispersed throughout the cytoplasm. In contrast, a dose-related reduction in the 119-kDa complex at the outer periphery of the cytoplasm was observed for virus-infected cells pretreated with 10^{-7} to 10^{-5} *M* Δ^9-THC. Depletion of 119 kDa determinants from the outer cytoplasm was accompanied by a corresponding concentration of these glycoproteins at the perinucleus. For virus-infected cells pretreated with 10^{-5} *M* drug, the 119-kDa complex was confined to the perinucleus. Light and electron microscopy revealed that the perinuclear 119-kDa glycoprotein in drug-treated cells was circumscribed by microvacuoles which extended to the outer periphery of the cytoplasm and imparted a "foamy" appearance. Immunoelectron microscopy confirmed, and extended, the characterization of the drug dose-related reductions in the cell surface expression of the 119-kDa determinants. In vehicle-treated, virus-infected cells numerous "tufts" of immunoenzyme staining, indicative of the typical distribution of 119-kDa surface determinants, were observed evenly dispersed along the plasma membrane. The number and intensity of these "tufts" were reduced for cells treated with 10^{-7} and 10^{-6} *M* drug. For cells treated with 10^{-5} *M* Δ^9-THC, little staining for the 119-kDa complex was observed on the plasma membrane. In spite of the drug-induced depletion of the 119-kDa components of the cell surface, electron microscopy revealed no qualitative differences in the expression of the 119-kDa glycoproteins on the envelopes of virus released from cells treated with Δ^9-THC (i.e., 10^{-5} to 10^{-7} *M*).

Discussion. Under the conditions described in this study, *in vitro* pretreatment of Vero cells for 24 hr with 10^{-7} to 10^{-5} *M* Δ^9-THC

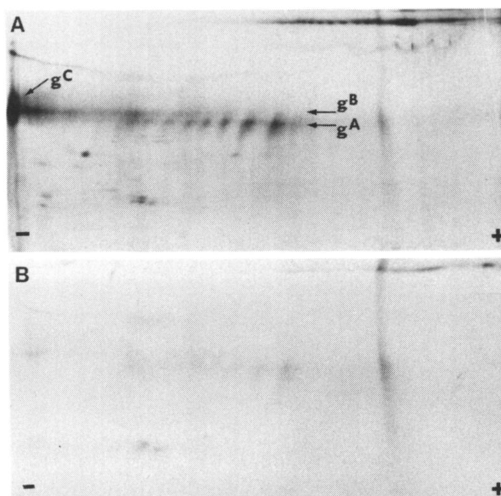

FIG. 3. Two-dimensional SDS–polyacrylamide gel analysis of the incorporation of [^{14}C]glucosamine into HSV2-specified cell-associated glycoproteins in cells pretreated with vehicle or with Δ^9-THC for 24 hr. (A) [^{14}C]Glucosamine incorporation into glycoprotein species of HSV2-infected cells pretreated with vehicle. (B) [^{14}C]Glucosamine incorporation into glycoprotein species of HSV2-infected cells pretreated with 10^{-5} *M* Δ^9-THC. Note the major reduction in the incorporation of glucosamine derivatives in all species of HSV2-specified glycoproteins.

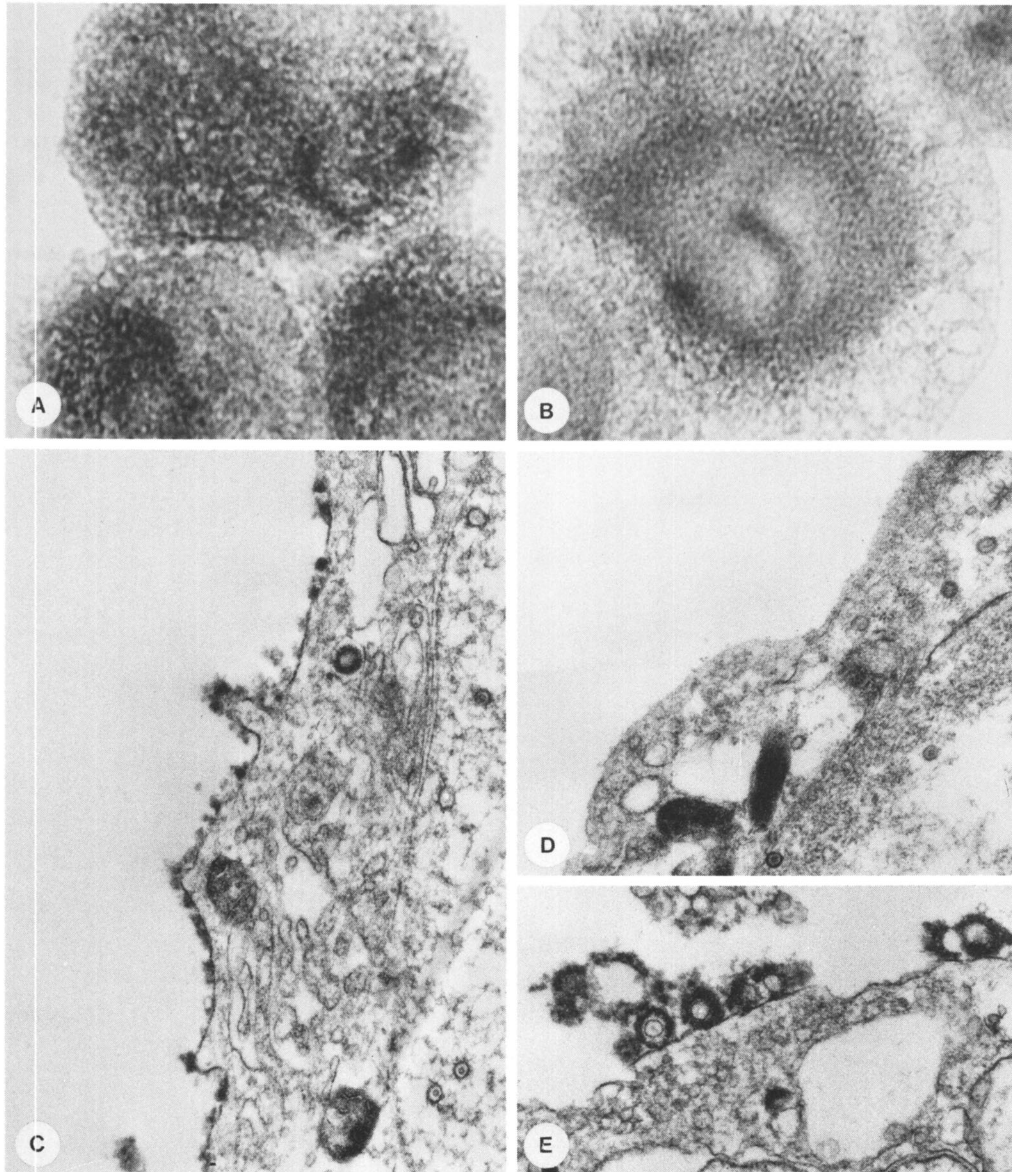

FIG. 4. Localization of the 119-kDa glycoprotein complex in HSV2-infected cells pretreated with vehicle or with Δ^9-THC for 24 hr. (A and B) Light microscopy immunoperoxidase staining for the intracellular 119-kDa determinants. Cells pretreated with vehicle (A) exhibit 119-kDa components throughout the cytoplasm in contrast to cells pretreated with 10^{-5} M Δ^9-THC (B) for which 119-kDa determinants are concentrated at the perinucleus (×825). Note the vacuolization at the outer periphery of the cytoplasm circumscribing the perinucleus in cells treated with Δ^9-THC. (C–E) Electron microscopy immunoperoxidase staining for the HSV2 119-kDa major envelope glycoprotein determinants on the plasma membrane and on the HSV2 envelope. Cells pretreated with vehicle (C) exhibit 119-kDa determinants uniformly along the plasma membrane in contrast to cells pretreated with 10^{-5} M drug (D) for which plasma membrane 119-kDa expression is absent (×27,750). Staining for 119-kDa determinants on the envelope of the HSV2 remains unaffected following treatment of cells with 10^{-5} M Δ^9-THC (E) (×28,125).

resulted in dose-related reductions in the synthesis and glycosylation of HSV2-specified glycoproteins and in inhibition of their transport to the plasma membrane. The suppression of glycoprotein synthesis was supported by the observed drug dose-related reductions in immunostaining for the 119-kDa HSV2 major envelope glycoprotein complex in Western blots of two-dimensional SDS–PAGE. The individual components of this complex exhibit relative molecular weights of 133, 111, and 105 kDa and correspond to gC, gB, and pgB, respectively (22–24). The role of gC *in vivo,* and its function in the maintenance of HSV2 infection within the host, is not fully understood although it has been shown that one of two monoclonal antibodies to this glycoprotein protected mice against lethal infection with HSV2 (25). The glycoprotein gB is a key glycoprotein involved in the initial stages of virus infection and may be requisite for virus penetration of the host cell (26). This glycoprotein induces high levels of neutralizing antibody and may play a significant role in protection against HSV infection. Balachandran *et al.* (25) reported that anti-gB monoclonal antibodies protected mice from lethal infection with HSV, although these antibodies failed to neutralize HSV *in vitro.* The pgB (i.e., gA) component has been shown to be a precursor of gB (24). Drug-induced reductions, also, were noted for the 92-, 65-, 54-, 51-, and 42-kDa glycoprotein species. The 92-kDa species corresponds to glycoprotein gE, the virus-induced Fc-binding protein (27), while the 54- and/or 51-kDa components may represent the glycoprotein gD (21). The glycoprotein gD has been reported to account for cross-neutralization between HSV1 and HSV2 (28). The decreases in virus-specified protein synthesis recorded for the present study are in agreement with reports that Δ^9-THC suppresses macromolecular synthesis in various mammalian culture systems (6, 29).

The decrease in glycoprotein synthesis was accompanied by a cannabinoid-induced alteration in glycosylation. These conclusions are supported by the observed depletion of acidic glycoprotein 119-kDa components following Western immunoblotting of two-dimensional SDS–PAGE. These observations were further substantiated by autoradiography of two-dimensional gels of [^{14}C]glucosamine-labeled HSV2-infected whole cell homogenates which demonstrated drug dose-related inhibition of incorporation of glucosamine derivatives into virus glycoprotein species. For cells pretreated with 10^{-5} *M* Δ^9-THC relatively little [^{14}C]glucosamine was incorporated into glycoprotein. This reduction in [^{14}C]glucosamine incorporation into the 119-kDa carbohydrate moiety was comparably greater than the reduction for 119-kDa protein biosynthesis noted by Western immunoblotting. These observations suggest that Δ^9-THC exerted maximal effect at the level of post-translational modification of these virus-specified macromolecules.

Δ^9-THC inhibited expression of HSV2 glycoproteins on the infected cell surface. These conclusions were supported by immunoperoxidase studies at the light and electron microscopy levels which demonstrated reduced expression of 119-kDa major envelope glycoprotein determinants on the plasma membrane of HSV2-infected cells treated with 10^{-7} or 10^{-6} *M* of the drug. Cells pretreated with 10^{-5} *M* Δ^9-THC exhibited little surface staining when compared to vehicle controls. The inhibition in surface expression of the 119-kDa components was accompanied by their depletion from the outer periphery of the cytoplasm and accumulation at the perinucleus. A similar pattern of internalization of HSV-specified glycoproteins has been reported for infected cells treated with 2-deoxyglucose, an antagonist of glycoprotein synthesis (30). This pattern of perinuclear accumulation was attributed to dysfunction in glycosylation of virus-specified gene products, possibly within the Golgi complex.

The Δ^9-THC-induced inhibition in HSV2 glycoprotein expression may be a consequence of perturbation of cytoplasmic and cell surface membranes. Δ^9-THC has been shown to be highly lipophilic (31, 32), to impart increased fluidity to membranes (33, 34), and to disrupt cell surface and intracytoplasmic membranes (7, 18, 35). Δ^9-THC, also, was shown in the present investigation to induce vacuolization, first at the outer periphery of the cytoplasm, and then extending into the inner cytoplasm to a degree depending on drug concentration and duration of exposure. Such membrane alterations could account for decreased synthesis, glycosylation, and transport of virus glycoproteins to the plasma membrane. Processing of immature, high mannose content

glycoproteins to complex species containing an increased concentration of glucosamine-derived carbohydrate residues has been observed to occur in the Golgi apparatus (27, 36). Thus, drug-induced disturbance of Golgi apparatus membranes could account for dysfunctional glycosylation, with consequent drug dose-related depletion of acidic virus-specified glycoproteins species.

In spite of the drug dose-related reductions in HSV2-specified, cell-associated glycoprotein, immunoperoxidase studies indicated that expression of the 119-kDa glycoprotein complex on virus envelopes was unaffected. Furthermore, 10^{-7} to 10^{-5} M Δ^9-THC treatment of cells did not affect the total yield of infectious virus (18). The paradox of drug-induced suppression of virus-specified, cell-associated glycoprotein concomitant with unaltered amounts of infectious virus may be resolved by proposing that Δ^9-THC differentially targets cellular surface and cytoplasmic membranes. Indeed, electron microscopy studies have shown that Δ^9-THC alters the membranous architecture of cells (G. A. Cabral *et al.*, submitted for publication). Drug-treated rat B103 neuroblastoma cells first exhibited vacuolization at the outer periphery of the cell. Vacuolization, then, progressed into the inner cytoplasm in direct relationship to the Δ^9-THC concentration in the culture medium and to the duration of drug exposure. The drug doses (i.e., 10^{-5} to 10^{-7} M) which effected these membrane alterations did not alter the total concentration of neuroblastoma cytoskeletal proteins (i.e., actin, tubulin). The observed reduction in HSV2 glycoprotein biosynthesis and glycosylation in the present study, therefore, suggests that the maturation and distribution of membrane-bound proteins are most susceptible to the lipophilic action of Δ^9-THC. The recorded perinuclear accumulation of the 119-kDa glycoprotein complex in drug-treated cells, accompanied by cytoplasmic microvacuolization circumscribing the perinucleus, further supports this hypothesis. Thus, Δ^9-THC may effect a depletion of the bulk of virus-specified cell-associated glycoprotein from the cell surface and from the cytoplasm while allowing for residual glycoprotein in the perinucleus for incorporation into viral envelopes. Since the HSV2 assembles in the nucleus and obtains its envelope from the inner nuclear membrane (37), it may remain relatively unaffected by Δ^9-THC at concentrations lower than 10^{-5} M.

The Δ^9-THC-induced *in vitro* inhibition of expression of HSV2-specified cell-associated glycoproteins may have an *in vivo* correlate. Δ^9-THC exposure may facilitate virus release in the infected host as a result of drug-induced membrane alteration and vacuolization (18). This enhanced release coupled with diminished expression of virus-specified cell surface glycoproteins which normally account for induction of cellular and humoral immunity (24) could contribute to diminished host resistance to herpes sequelae.

The authors thank Ms. Judy Williamson for excellent technical assistance and Dr. L. Harris, Chairman of the Department of Pharmacology and Toxicology, for kindly providing Δ^9-tetrahydrocannabinol. This research was supported by Grant R0I DAO 3647 from the National Institute of Drug Abuse and by Biomedical Research Support Grants 2S07 RR05724, 2S07 RR05430, and 2S07 RR05697 to Virginia Commonwealth University.

1. Nahas GG, Sucia-Foca N, Armand SP, Morishima A. Inhibition of cellular mediated immunity in marijuana smokers. Science **183**:419–420, 1974.
2. Holsapple MP, McNerney PJ, Mishkin EM, Cabral GA. In vivo and in vitro effects of Δ^9-tetrahydrocannabinol on immune responsiveness and resistance to vaginal herpes simplex virus type II (HSV2) infection by B6C3F1 mice. Proceedings of the Oxford Symposium on Cannabis. Oxford, IRL Press, pp439–450, 1985.
3. Morahan PS, Klykken PC, Smith SH, Harris LS, Munson AE. Effects of cannabinoids on host resistance to *Listeria monocytogenes* and herpes simplex virus. Infect Immun **23**:670–674, 1979.
4. Blevins RD, Regan JD. Δ^9-tetrahydrocannabinol: Effect on macromolecular synthesis in human and other mammalian cells. Arch Toxicol **35**:127–135, 1976.
5. Nahas GG, Morishima A, Desoize B. Effect of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. Fed Proc **36**:1748–1752, 1977.
6. Desoize B, Leger C, Nahas GG. Plasma membrane inhibition of macromolecular precursor transport by THC. Biochem Pharmacol **28**:1113–1118, 1979.
7. Cabral GA, McNerney PJ, Mishkin EM. Effect of micromolar concentrations of Δ^9-tetrahydrocannabinol on herpes simplex virus type 2 replication *in vitro*. J Toxicol Environ Health, in press.
8. Courtney RJ. Virus specific components of herpes simplex virus involved in the immune response. In: Rouse BT, Lopez C, Eds. Immunobiology of Herpes Simplex Virus Infection. Boca Raton, FL, CRC Press, pp33–44, 1984.

9. Hamelin C, Chagnon A, Farwel M. Identification of atypical strains of herpes simplex virus using a simplified DNA finger printing method. Microbiol Immunol **28**:723–729, 1984.
10. Rapp F. Variants of herpes simplex virus: Isolation, characterization, and factors influencing plaque formation. J Bacteriol **86**:985–991, 1963.
11. Ornstein L. Disc electrophoresis. I. Background and theory. Ann NY Acad Sci **121**:321–349, 1964.
12. Davis BJ. Disc electrophoresis. II. Method and application to human serum proteins. Ann NY Acad Sci **121**:404–427, 1964.
13. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem **250**:4007–4021, 1975.
14. Symington J. Electrophoretic transfer of proteins from two-dimensional gels to sheets and their detection. In: Celis JE, Bravo R, Eds. Two-Dimensional Gel Electrophoresis: Methods and Applications. New York, Academic Press, pp127–168, 1984.
15. Flannery VL, Courtney RJ, Schaffer PA. Expression of an early non-structural antigen of herpes simplex virus in cells transformed in vitro by herpes simplex virus. J Virol **21**:284–291, 1977.
16. Cabral GA, Marciano-Cabral F, Fry D, Lumpkin CK, Mercer L, Goplerud D. Expression of herpes virus type 2 antigens in premalignant and malignant vulvar cells. Amer J Obstet Gynecol **143**:611–619, 1982.
17. Mollenhauer HH. Plastic embedding mixtures for use in electron microscopy. Stain Technol **38**:111–114, 1964.
18. Cabral GA, McNerney PJ, Mishkin EM. Δ^9-tetrahydrocannabinol enhances release of herpes simplex virus type 2. J Gen Virol **67**:2017–2022, 1986.
19. Baucke RB, Spear PG. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J Virol **32**:779–789, 1979.
20. Killington RA, Powell KL. Growth, assay, and purification of herpes viruses. In Mahy WJ, Ed. Virology: A Practical Approach. Oxford, IRL Press, pp207–236, 1985.
21. Spear PG. Membrane proteins specified by herpes simplex virus. I. Identification of four glycoprotein precursors and their products in type-1-infected cells. J Virol **17**:991–1008, 1976.
22. Zweig M, Showalter SD, Bladen SV, Heilman CJ Jr, Hampar B. Herpes simplex virus type 2 glycoprotein gF and type 1 glycoprotein gC have related antigenic determinants. J Virol **47**:185–192, 1983.
23. Para MF, Zezulak KM, Conley AJ, Weinberger M, Snitzer K, Spear PG. Use of monoclonal antibodies against two 75,000-molecular-weight glycoproteins specified by herpes simplex virus type 2 in glycoprotein identification and gene mapping. J Virol **45**:1223–1227, 1983.
24. Eberle R, Courtney RJ. Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex type 1. J Virol **35**:902–917, 1980.
25. Balachandran N, Bacchetti S, Rawls WE. Protection against lethal challenge of BALB/c mice by passive transfer of monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. Infect Immun **37**:1132–1137, 1982.
26. Little SP, Jofre JT, Courtney RJ, Schaffer PA. A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. Virology **115**:149–160, 1981.
27. Spear PG. Glycoproteins specified by HSV. In: Roizman B, Ed. The Herpesviruses. New York, Plenum, pp315–356, 1985.
28. Dix RD, Pereira L, Baringer JR. Use of monoclonal antibody directed against herpes simplex virus glycoproteins to protect mice against acute virus-induced neurological disease. Infect Immun **34**:192–199, 1981.
29. Mon JJ, Jansing RL, Doggett S, Stein JL, Stein GS. Influence of Δ^9-tetrahydrocannabinol on cell proliferation and macromolecular synthesis in human cells. Biochem Pharmacol **21**:1759–1765, 1978.
30. Dix R. Herpes simplex virus glycoprotein synthesis and the role of virus-specific glycoproteins during virus morphogenesis. Doctoral dissertation, Baylor College of Medicine, Houston, TX, 1978.
31. Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC. 1978. Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. Proc Natl Acad Sci **75**: 2458–2462, 1978.
32. Poddar MK, Mitra G, Ghosh JJ. Δ^9-Tetrahydrocannabinol-induced changes in brain ribosomes. Toxicol Appl Pharmacol **46**:737–757, 1978.
33. Howlett AC, Fleming RM. Cannabinoid inhibition of adenylate cyclase: Pharmacology of the response in neuroblastoma cell membranes. Mol Pharmacol **26**:532–538, 1984.
34. Wing DR, Leuschner JTA, Brent GA, Harvey DJ, Paton WDM. Quantification of in vivo membrane-associated Δ^1-tetrahydrocannabinol and its effect on membrane fluidity. Proceedings of the Oxford Symposium on Cannabis. Oxford, IRL Press, pp85–92, 1985.
35. Issidorides MR. Cannabis and arginyl proteins. Proceedings of the Oxford Symposium on Cannabis. Oxford, IRL Press, pp411–418, 1985.
36. Johnson DC, Spear PG. O-linked oligosaccharides are acquired by herpes simplex glycoproteins in the Golgi apparatus. Cell **32**:987–997, 1983.
37. Srinivas RV, Balachandran N, Alonso-Caplen FV, Compans RW. Expression of herpes simplex virus glycoproteins in polarized epithelial cells. J Virol **58**: 689–693, 1986.

Received October 28, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted January 13, 1987.