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Inhibition of Sindbis virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat-shock protein synthesis

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Summary

Cyclopentenone prostaglandins (PGs) have been shown to inhibit the replication of several DNA and RNA viruses. Here we report on the effect of prostaglandin A_1 (PGA_1) on the multiplication of a positive strand RNA virus, Sindbis virus, in Vero cells under one-step multiplication conditions. PGA_1 was found to inhibit Sindbis virus production dose-dependently, and virus yield was reduced by more than 90% at the concentration of 8 μg/ml, which was non-toxic to the cells and did not inhibit DNA, RNA or protein synthesis in Vero cells. The cyclopentenone prostaglandin Δ^{12}-PGJ_2 was also shown to be a potent inhibitor of Sindbis virus replication. Virus-induced reduction of [^{3}H]uridine uptake by cells was partially prevented by PGA_1 treatment, which also caused a 1 h delay in the peak of virus RNA synthesis. SDS-PAGE analysis of [^{35}S]methionine-labeled proteins showed that PGA_1 moderately inhibited the synthesis of the viral structural proteins E1, E2 and C, and induced the synthesis of a 72 kDa M_r protein, identified as a heat-shock protein related to the HSP70 group, in both virus-infected and uninfected cells. Actinomycin D treatment completely prevented PGA_1-antiviral activity, indicating that a cellular product is responsible for this action. PGA_1-induced HSP70 is a good candidate for this role.

Sindbis virus; Prostaglandin; Cyclopentenone; Heat-shock protein; Actinomycin D

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Introduction

Prostaglandins (PGs) are a class of naturally occurring cyclic 20-carbon fatty acids, synthesized by most types of eukaryotic cells from polyunsaturated fatty acid precursors (reviewed by Samuelsson, 1982). These compounds function as microenvironmental hormones and intracellular signal mediators, and participate in the regulation of a large variety of physiological and pathological processes, including cell proliferation and differentiation (Garaci et al., 1987), inflammation (Vane, 1987), immune response (Ninnemann, 1988), cytoprotection (Robert, 1981), the pathology of fever (Dinarello and Wolff, 1982) and virus replication (Santoro et al., 1990).

Viral transformation can affect PG biosynthesis in cultured cells, either increasing or decreasing it, depending on the virus model and the cell type. On the other hand, PGs have been shown to influence virus replication in cultured cells, but their effect varies in relation to the structure of the molecule, as well as the type of virus and host cells (reviewed by Santoro et al., 1990).

PGs of the E series inhibit the replication of measles virus in Vero cells (Dore-Duffy, 1982), of Mengo virus, MM virus and polio virus in L929 fibroblasts and HeLa cells (Giron, 1982), and of parainfluenza virus type 3 in WISH cells (Luczak et al., 1975). On the other hand, PGEs enhance the replication of herpes simplex virus (HSV) in Vero cells (Harbour et al., 1978), but have no effect on HSV multiplication in human fibroblasts (Trofatter and Daniels, 1980).

Prostaglandins of the A and J type, characterized by the presence of an α,β-unsaturated carbonyl group in the cyclopentane ring (cyclopentenone PGs) (Fig. 1A), have been shown to inhibit the replication of several DNA and RNA viruses. The mechanism of antiviral action of prostaglandins has been previously studied in DNA or negative strand-RNA viruses, and the inhibition of virus replication has been associated with specific alterations in the synthesis and/or maturation of specific virus proteins (reviewed by Santoro et al., 1990).

In the present report, we have studied the effect of PGA_1 and Δ^{12}-PGJ_2 (9-deoxy-Δ^9,Δ^{12}-13,14 dihydro-PGD_2, a natural dehydration product of prostaglandin D_2) on the multiplication of Sindbis virus (SNV), an enveloped, ss(+)RNA virus, belonging to the Togaviridae family. The 5′ two-thirds of the viral genome encode for four nonstructural proteins, which function as replicase-transcriptase, while the 3′ one-third encodes for the two structural glycoproteins E1 and E2, and the non-glycosylated capsid protein C. These proteins are translated from a subgenomic 26S RNA as a polyprotein that is cleaved post-translationally to form five polypeptides. The smallest proteins, E3 and 6K, probably serve as signal sequences and are not found in the mature virion (Strauss and Strauss, 1986).

The results described in the present study demonstrate that *Togaviruses* are sensitive to the antiviral activity of cyclopentenone prostaglandins, and that the antiviral activity is a cell-mediated event, associated with the induction of heat-shock protein synthesis.

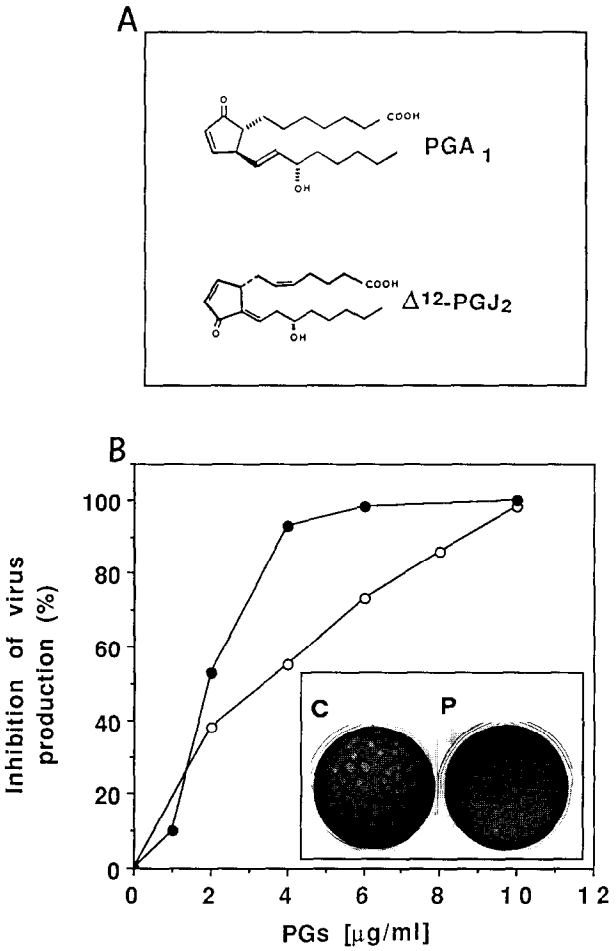

Fig. 1. Dose-dependent inhibition of Sindbis virus replication by prostaglandins. (A) Structure of PGA_1 and Δ^{12}-PGJ_2. (B) Vero cells were treated with different concentrations of PGA_1 (○) or Δ^{12}-PGJ_2 (●), soon after virus infection. Virus titers were determined at 8 h p.i. Each point represents the mean of duplicate samples. The picture in the insert shows the reduction in the size of virus plaques at 8 h p.i., when PGA_1 (10 μg/ml) (P) or control diluent (c) were added directly into the agar during plaque assay.

Materials and Methods

Cell cultures

Vero cells were grown at 37°C in a 5% CO_2 atmosphere in Eagle's minimal essential medium (MEM) supplemented with 6% fetal calf serum (FCS), 2 mM glutamine, 100 IU/ml penicillin G and 100 μg/ml streptomycin.

Virus infection and prostaglandin treatment

Sindbis virus AR 339 was propagated in Vero cell monolayers at a multiplicity of 0.1 PFU/cell in MEM containing 2% FCS (maintenance

medium). After incubation for 24 h at 37°C, the supernatant was collected and clarified by centrifugation at 10 000 × *g* for 10 min. Stock virus was stored in small aliquots at −80°C. For virus infection, confluent monolayers of Vero cells grown in 24-well plates were inoculated with 0.2 ml of Sindbis virus (3 PFU/cell). After a 1 h incubation at 37°C, the viral inoculum was removed. Cell monolayers were washed three times with phosphate-buffered saline (PBS), and incubated with 1 ml of MEM, supplemented with 2% FCS, in the presence of prostaglandins or control diluent. PGA_1 and Δ^{12}-PGJ_2 (Cayman) were stored as a 100% ethanolic stock solution (10 mg/ml) at −20°C, and were diluted to the appropriate concentration at the time of use. Control media contained the same concentration of ethanol diluent, which was shown not to affect cell metabolism. Virus production in untreated- and PG-treated cultures was determined by plaque assay, 8 h after infection.

Plaque assay

Serial 10-fold dilutions of Sindbis virus were inoculated on confluent Vero cell monolayers. After a 1 h adsorption period at 37°C, the inoculum was removed and cells were washed three times with PBS, before the addition of MEM containing 2% FCS and 1 Seaplaque agarose (Miles). After 2 days of incubation at 37°C in a 5% CO_2 atmosphere, plaques were stained with 0.33% neutral red solution.

DNA, RNA and protein metabolism

DNA, RNA and protein synthesis were determined in confluent monolayers of uninfected or virus-infected Vero cells after treatment with PGA_1 (8 μg/ml), Δ^{12}-PGJ_2 (4 μg/ml), or control diluent. Cells were labeled for a period of 8 h starting soon after Sindbis virus infection with 5 μCi/ml/2.8 × 10^5 cells of [^{3}H]thymidine, [^{3}H]uridine or [^{35}S]methionine (Amersham) for DNA, RNA or protein synthesis, respectively, and the radioactivity incorporated into acid-soluble and -insoluble material was determined. Briefly, cells were washed three times with PBS, and 0.4 ml 5% TCA was added to each culture. After 1 h, radioactivity of acid-soluble material was determined. Acid-insoluble radioactivity was measured after washing three times the TCA precipitates with ethanol, drying under an infrared lamp and dissolving the samples in 0.4 ml of a solution containing 0.1 M NaOH, 0.5% SDS.

Protein synthesis and PAGE analysis

Confluent Vero cell monolayers were labeled with [^{35}S]methionine (5 μCi/ml/2.8 × 10^5 cells) for 3 h, starting at 5 h p.i. Cells were usually preincubated 15 min in methionine-free medium containing 2% dialyzed FCS. After labeling, cells were washed, lysed in lysis buffer (2% SDS, 10% glycerol, 0.001% Bromophenol blue, 0.1 M dithiothreitol, 0.0625 M Tris-HCl pH 6.8) and the radioactivity incorporated was determined. Samples were analyzed by SDS-PAGE in a vertical slab apparatus (3% stacking gel, 10% resolving gel) using the buffer system described by Laemmli (1970). Gels were washed, fixed in 10%

acetic acid, 10% TCA and 30% methanol, dried under vacuum and autoradiographed using Kodak films (Eastman Kodak). Quantitative determination of protein synthesis was measured by densitometric analysis of autoradiographic patterns, using a laser-beam densitometer (Ultroscan XL, LKB Instruments, Rockville, MD). The M_rs of polypeptides were calculated by comparison with the following markers: myosin (200 kDa), phosphorylase b (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa) and lysozyme (14.3 kDa).

Immunoblot analysis

For immunoblot analysis, an equal amount of protein from each sample was separated by SDS-PAGE, as described above, and blotted onto nitrocellulose using the technique described by Burnette (1981). After transfer, the filters were incubated with an anti-72/73 kDa HSP monoclonal antibody (MAb) (diluted 1:500) from HeLa cells (Amersham), which has been shown to be reactive against human and monkey HSP70, in TEN-Tween 20 buffer (0.05 M Tris-HCl (pH 7.4), 5 mM EDTA, 0.15 M NaCl, 0.05% Tween 20), and the bound antibody was detected using horseradish peroxidase-linked sheep antimouse antibody (Amersham). Molecular weights were calculated using Bio-Rad low M_r markers.

Statistical analyses

Statistical analyses were performed using the Student's *t*-test for unpaired data. Data are expressed as the mean $\pm$ S.D.; P values of <0.05 were considered significant.

Results

Effect of prostaglandins on Sindbis virus replication

The effect of PGA_1 and Δ^{12}-PGJ_2 on Sindbis virus replication in Vero cells was studied under one-step multiplication conditions. After viral adsorption (1 h, 37°C, 3 PFU/cell) the drugs or control diluent were added to the culture medium and viral yield was determined 8 h post-infection (p.i.) by plaque assay. As shown in Fig. 1B, both PGs were found to inhibit virus production dose-dependently, Δ^{12}-PGJ_2 being more effective. An inhibition of approx. 1 log was obtained at a concentration of 8 μg/ml and 4 μg/ml for PGA_1 and Δ^{12}-PGJ_2 respectively.

In order to determine whether PGs could influence the cell-to-cell spreading of virus infection, Vero cell monolayers were infected with serial SNV dilutions. After the 1 h infection period, virus inoculum was removed and medium containing 2% FCS, 1% Seaplaque agarose and PGA_1 (8 μg/ml) or control diluent was added. At 48 h p.i., the number of plaques was determined, after neutral red staining. No significant decrease in the number of plaques was found, indicating that PGA_1 treatment did not affect virus entry or early stages

of virus infection. However, PGA_1 treatment caused a dramatic reduction in the plaque size, indicating an inhibition of cell-to-cell spreading of virus infection. Approx. 100 plaques were measured for each sample, and divided into three categories on the basis of their diameter: plaques with diameter >1.5 mm (89% in control; 16% in PGA_1-treated samples); plaques with diameter between 0.6 mm and 1.5 mm (11% in control, 46% in PGA_1-treated samples); non-measurable plaques with diameter <0.6 mm (0 in control, 38% in PGA_1-treated samples). The average diameter of the measurable size plaques was decreased by more than 60% in PGA_1-treated samples as compared to control (Fig. 1, insert).

Effect of PGs on host cell macromolecular synthesis

Under the conditions described above, both PGs were shown not to be toxic to uninfected cultures, as demonstrated by microscopic examination and vital

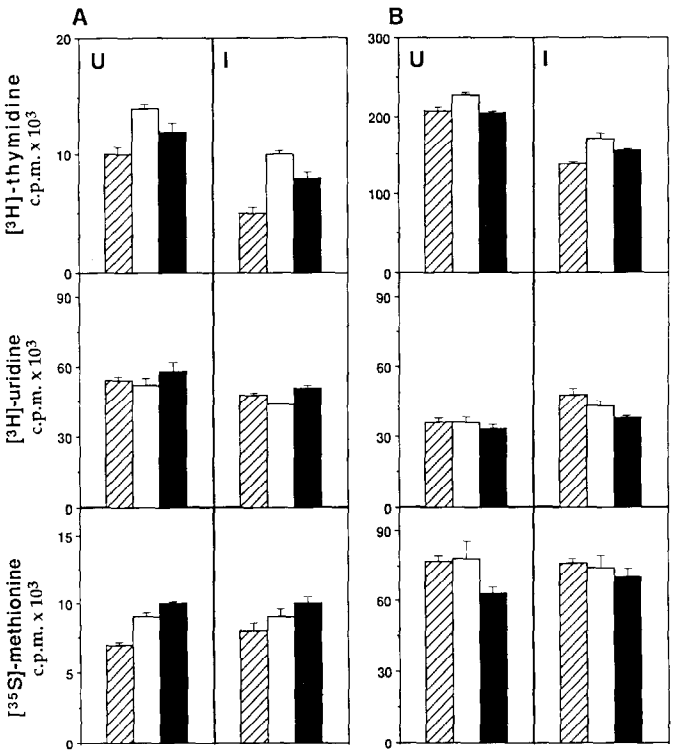

Fig. 2. Effect of prostaglandins on macromolecular synthesis in uninfected or virus-infected Vero cells. DNA, RNA and protein synthesis were measured in confluent monolayers of cells infected or mock-infected and treated with PGA_1 (8 μg/ml), Δ^{12}-PGJ_2 (4 μg/ml) or control diluent. Cells were labeled starting soon after infection for the following 8 h with 5 μCi/ml/2.8 $\times$ 10^5 cells of [^{3}H]thymidine, [^{3}H]uridine or [^{35}S]methionine. (A) Uptake of precursors; (B) Incorporation of precursors into DNA, RNA or proteins in uninfected (U) or Sindbis virus infected (I) cells. Data represent the mean $\pm$ S.D. of duplicate samples. (▨), Untreated; (□), PGA_1; (■), Δ^{12}-PGJ_2.

dye uptake. The effect of PGA_1 (8 μg/ml) and Δ^{12}-PGJ_2 (4 μg/ml) on macromolecular synthesis was studied in both uninfected and SNV-infected Vero cells. In uninfected cells, (Fig. 2) an 8 h treatment with PGs at 37°C did not suppress either the uptake by the cells (Fig. 2A) or the incorporation into macromolecules (Fig. 2B) of [^{3}H]uridine or [^{35}S]methionine. [^{3}H]Thymidine and [^{35}S]methionine uptake were slightly increased, while there was no significant change in DNA or protein synthesis. In Vero cells infected with Sindbis virus, both PGA_1 and Δ^{12}-PGJ_2 caused changes similar to those observed in uninfected cells (Figs. 2A,B).

Effect of PGA_1 on RNA synthesis in Sindbis virus infected cells

In order to determine the effect of PGA_1 on RNA synthesis after Sindbis virus infection, confluent monolayers of infected or uninfected Vero cells treated with PGA_1 (8 μg/ml) or control diluent were labeled with 3 μCi/ml of [^{3}H]uridine (60 min pulses) at different times after infection, and both the uptake of precursor by the cells and the incorporation into RNA were determined, as previously described. PGA_1 treatment partially prevented the virus-induced reduction of [^{3}H]uridine uptake by the cells (Fig. 3A) and the virus-mediated inhibition of RNA synthesis at 8 h p.i. (Fig. 3B). PGA_1 caused a slight delay in the peak of RNA synthesis induced by the virus 4–5 h p.i., but it did not suppress it, indicating that PGA_1 does not dramatically reduce viral RNA synthesis.

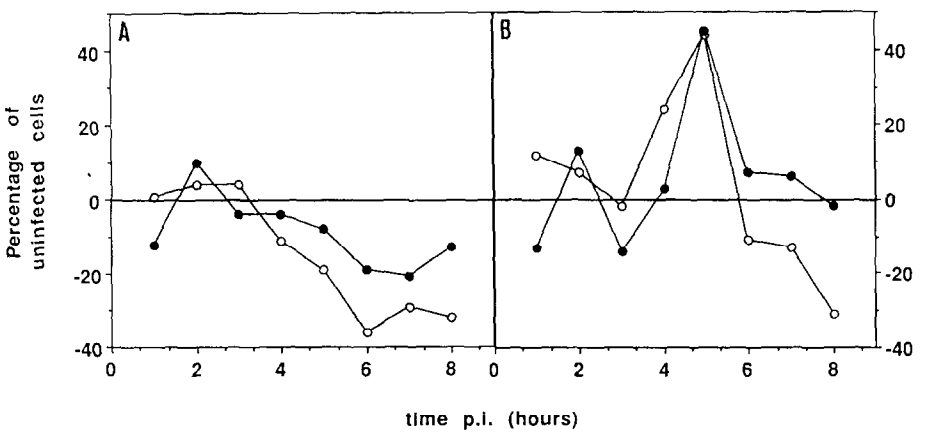

Fig. 3. Effect of PGA_1 on the uptake of uridine and synthesis of RNA in Sindbis virus-infected Vero cells. Monolayers of Vero cells were infected with Sindbis virus (3 PFU/cell) and treated with PGA_1 (8 μg/ml) or control diluent. Cells were labeled (60 min pulses) with 3 μCi/ml of [^{3}H]uridine, at different times p.i. Results are expressed as percent of uptake (A) or incorporation (B) in uninfected cells at that time. For each point, data represent the mean of duplicate samples. (○), Control; (●), PGA_1. Values of [^{3}H]uridine uptake and incorporation into RNA in uninfected cells (time 0) were: uptake, C = 17.31 ± 1.41; PGA_1 = 17.42 ± 0.17 c.p.m. × $10^3/10^5$ cells; incorporation, C = 3.24 ± 0.10; PGA_1 = 3.11 ± 0.07 c.p.m. × $10^3/10^5$ cells.

Effect of PGA_1 on protein synthesis in uninfected and Sindbis virus infected cells

To determine whether PGA_1 treatment could alter cellular protein synthesis, confluent monolayers of Vero cells were treated with different concentrations of PGA_1 and labeled with [^{35}S]methionine for 3 h, starting 5 h after PGA_1 treatment. After labeling, the radioactivity incorporated into TCA-insoluble material was determined and cell extracts were separated by SDS-PAGE and processed for autoradiography. Results shown in Fig. 4A demonstrate that although PGA_1-treatment did not alter the general pattern of protein synthesis in uninfected Vero cells, it induced the production of a 72 kDa (p72) cellular protein. Densitometric analysis of autoradiographic patterns showed that p72 synthesis, minimal in control cells, increased dose-dependently in response to PGA_1 (Fig. 4B). The synthesis of a 68 kDa protein was also induced in uninfected cells by PGA_1 at the highest concentration (Fig. 4A). To determine

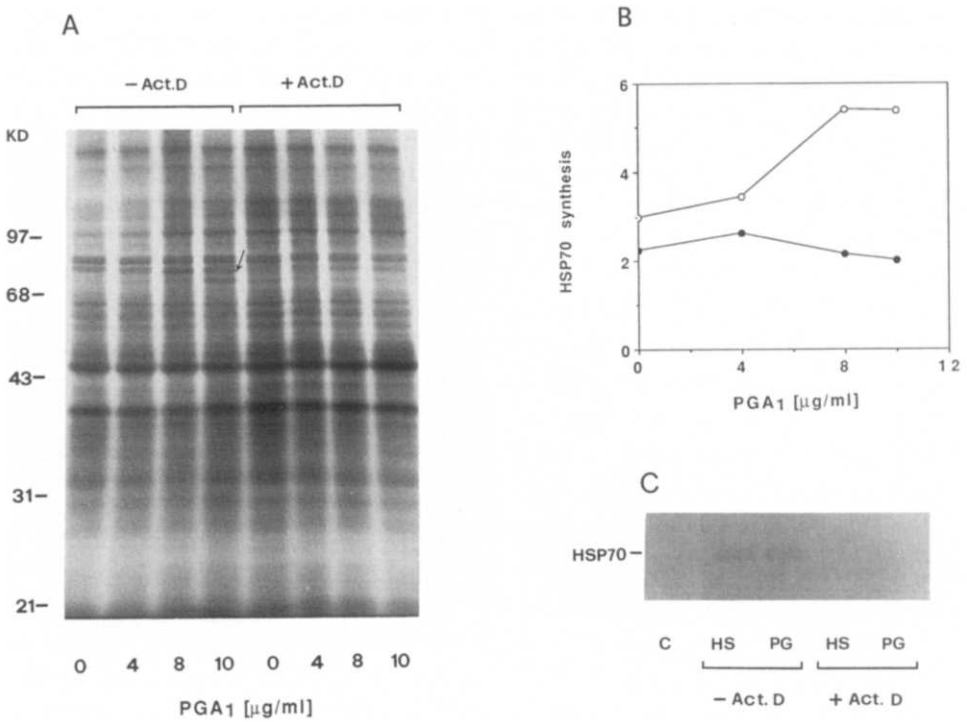

Fig. 4. Effect of PGA_1 on Vero cell protein synthesis. (A) SDS-PAGE analysis and autoradiography of polypeptides synthesized in Vero cells after an 8 h treatment with different concentrations of PGA_1 or control diluent in the presence or absence of actinomycin D (2 μg/ml). Cells were labeled with 5 μCi/well of [^{35}S]methionine, (3 h pulse), starting 5 h after PGA_1 treatment. The arrow indicates the position of the 72 kDa protein induced in PGA_1-treated cells. (B) Quantitative determination of HSP70, as measured by densitometric analysis, is expressed as percentage of total proteins in cells treated with different concentrations of PGA_1 (○), or PGA_1 + actinomycin D (●). (C) Identification of p72 as a HSP by immunoblot analysis. Proteins from control (37°C) (C), heat shocked (45°C for 20 min) (HS) or PGA_1-treated (10 μg/ml) (PG) Vero cells in the presence or the absence of actinomycin D (2 μg/ml) were separated by SDS-PAGE and processed for immunoblot analysis using anti 72/73 kDa HSP MAbs.

whether PGA_1 was acting on RNA transcription or translation, actinomycin D (2 μg/ml) was added to Vero cells in the same experimental conditions. Treatment with this drug totally prevented the induction of the 72 kDa cellular protein, indicating that PGA_1 is acting by inducing the expression of a cellular gene (Figs. 4A,B). To investigate whether the PGA_1-induced p72 could be identified as a heat-shock protein (HSP), Vero cell monolayers were either heat-shocked (45°C for 20 min) or kept at 37°C, or kept at 37°C and treated with PGA_1 (8 μg/ml) for 24 h. After this time, cells were lysed and equal amounts of protein from each sample were separated by SDS-PAGE and processed for immunoblot analysis, using monoclonal antibodies against the human 72–73 kDa HSP from HeLa cells. Immunoblot analysis revealed the presence of a unique band of approx. M_r 72 kDa in heat-shocked and in PGA_1-treated Vero cells, identifying p72 as an HPS70 (Fig. 4C). Actinomycin D completely suppressed PGA_1-mediated HSP70 induction.

To study the effect of PGA_1 treatment on protein synthesis in Sindbis virus-infected cells, Vero monolayers were treated with different concentrations of PGA_1 or control diluent, soon after infection, and were labeled with [^{35}S]methionine for 3 h, starting at 5 h p.i. SDS-PAGE analysis of labeled proteins showed a dose-dependent increase in HSP70 levels (Figs. 5A,C), as shown also in uninfected cells (Figs. 4A,B,C). Fig. 5 also shows that PGA_1 treatment moderately inhibited the synthesis of the virus structural proteins E1, E2 and C (Figs. 5A,D), in a dose-dependent manner. However, no difference in virus protein synthesis was found at a concentration of 4 μg/ml, which inhibited virus production by approx. 50% of control; also the moderate decrease in virus protein synthesis shown at the highest concentration of PGA_1 (10 μg/ml) did not appear to be sufficient by itself to cause the dramatic inhibition of virus production obtained at this dose.

Suppression of PGA_1-antiviral effect by actinomycin D

Inhibition of virus protein synthesis by PGA_1 appeared to be inversely correlated with HSP70 synthesis (Figs. 5C,D). Since induction of HSP70 by PGA_1 treatment was suppressed by actinomycin D (Fig. 4), we investigated the effect of this compound on SNV production after PGA_1 treatment. Vero cell monolayers were infected with Sindbis virus and treated with PGA_1 (10 μg/ml) or ethanol control in the presence or the absence of actinomycin D (2 μg/ml). Fig. 5B shows that actinomycin D completely prevented inhibition of virus replication by PGA_1, demonstrating that cellular RNA synthesis is necessary for the antiviral activity to occur, and that PGA_1 action is cell-mediated. Moreover, actinomycin D treatment also suppressed both the induction of HSP70 synthesis (Figs. 4B,C and Fig. 5C) and the inhibition of virus protein synthesis (Fig. 5D) caused by PGA_1 treatment, suggesting that these two events could be correlated.

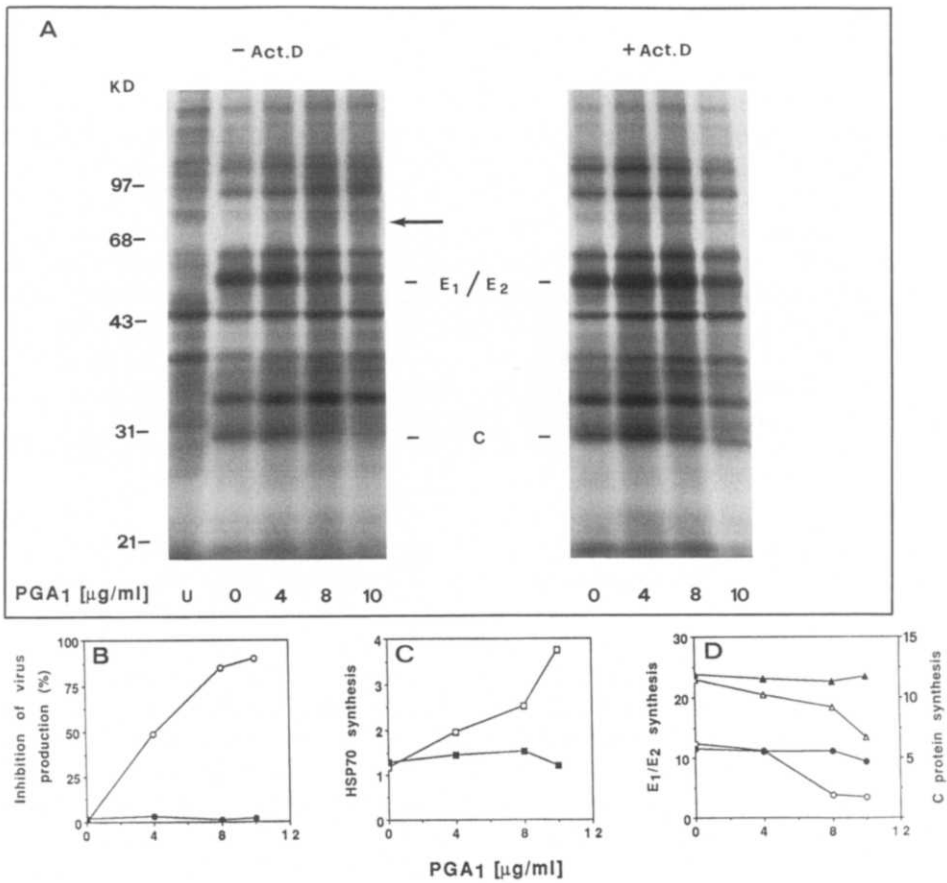

Fig. 5. Effect of PGA_1 and actinomycin D on Sindbis virus replication and protein synthesis. Vero cells uninfected or infected with SNV were treated with PGA_1, actinomycin D or ethanol control and labeled as described in the legend to Fig. 4. (A) SDS-PAGE analysis and autoradiography of uninfected (U) or SNV-infected cells treated with 0, 4, 8 or 10 μg/ml PGA_1 in the presence or the absence of actinomycin D (2 μg/ml). Arrow indicates the position of the 72 kDa cellular protein induced in PGA_1-treated cells. The viral envelope glycoproteins E1 and E2 and capsid protein C are indicated. Effect of different concentrations of PGA_1 on viral yield (B), HSP70 synthesis (C) and viral protein synthesis (D) in the presence (●,■,▲) or absence (○,□,△) of actinomycin D. Quantitative determination of HSP70 (□,■) and virus proteins E1/E2 (△,▲), and C (○,●) synthesis, as measured by densitometric analysis, is expressed as percentage of total protein synthesis. Treatment with actinomycin D completely prevented the antiviral activity of PGA_1 as well as HSP70 induction and virus protein synthesis inhibition.

Discussion

Starting from the early observation that prostaglandins of the A type inhibit the replication of a *Paramyxovirus* (Sendai virus) and can prevent the establishment of a persistent infection by this virus in African green monkey kidney cells (Santoro et al., 1980), the antiviral activity of prostaglandins of the A type and, more recently, of PGs of the J type has now been described for

several RNA and DNA viruses including *Orthomyxoviruses* (Santoro et al., 1988), picornaviruses (Ankel et al., 1985), rhabdoviruses (Santoro et al., 1983; Bader and Ankel, 1990), poxviruses (Santoro et al., 1982), herpesviruses (Yamamoto et al., 1989) and retroviruses (Ankel et al., 1991; D'Onofrio et al., 1990) growing in different types of cells. A long-acting synthetic analog of PGA_2 was also shown to suppress influenza A virus replication in mice (Santoro et al., 1988). The antiviral action is specific for PGs of the A and J type, while PGs of the B, E, F series, prostacyclin, 6-keto $PGF_{1\alpha}$, and thromboxane B_2 are inactive. This antiviral action is dose-dependent and can suppress virus production at non-toxic doses, which do not cause significant changes in the uptake of precursors or in the synthesis of DNA, RNA and proteins in uninfected cells. In several virus-cell systems studied, PG antiviral activity appears to be associated with alterations in the synthesis and/or maturation of specific virus proteins (Santoro et al., 1990).

In the case of Sendai virus, PGA_1 altered the glycosylation of the virus proteins HN and F_0, preventing the insertion of the HN glycoprotein in the host cell membrane and inhibiting virus maturation (Santoro et al., 1989b). Δ^{12}-PGJ_2 has also been recently shown to inhibit HN and F_0 glycosylation in Sendai virus-infected monkey kidney cells (Amici et al., 1992a). In mouse L fibroblasts, PGA_1 treatment was shown instead to specifically prevent the synthesis of three vaccinia virus (VV) polypeptides (Santoro et al., 1982). When cytoplasmic RNAs from PGA-treated VV-infected cells were translated in cell-free systems, similar selective inhibition of viral polypeptides was observed, but PGA_1, even at much higher doses, did not exert any direct inhibitory action on transcription in vitro, as measured in two cell-free systems, and had no effect on primary transcription-translation of VV RNAs when assayed in a coupled cell-free system, suggesting that the synthesis and/or activation of a host product was mediating the antiviral action (Benavente et al., 1984). On the other hand, it has been recently shown that PGA_1, at much higher concentrations, could inhibit in vitro the primary transcription of a negative strand RNA virus, vesicular stomatitis virus (Bader and Ankel, 1990).

In the present report we investigated the effect of PGA_1 on the replication of Sindbis virus, a positive-strand RNA virus, whose genome can function directly as mRNA for the translation of non-structural proteins in uninfected cells, and whose replication is not affected by the RNA synthesis inhibitor actinomycin D. PGA_1 was found to inhibit SNV production dose-dependently. Dramatic inhibition of virus replication could be obtained at doses of PGA_1 which did not inhibit either the uptake of precursors or the synthesis of DNA, RNA or protein in both uninfected or SNV-infected Vero cells. Similar results were obtained with the cyclopentenone prostaglandin Δ^{12}-PGJ_2. PGA_1 treatment also caused a dose-dependent reduction in the synthesis of the SNV structural proteins E1, E2 and C. However, the fact that considerable amounts of protein were synthesized at doses which suppressed virus production by more than 90%, and no inhibition of virus protein synthesis was found at a concentration

of 4 μg/ml which still inhibited virus replication by more than 50%, suggests that PGA_1 could be also acting by blocking a late event in virus maturation or virus assembly. This hypothesis is also supported by the fact that addition of PGA_1 in a late phase of the virus replication cycle (5 h p.i.) still resulted in a substantial inhibition of virus production (Mastromarino et al., data not shown).

Actinomycin D treatment completely prevented the PGA_1-mediated inhibition of virus protein synthesis as well as the block of virus replication, demonstrating that the antiviral activity of prostaglandins is a cell-mediated event.

The results described in this paper also show that PGA_1 treatment induces the synthesis of a 70 kDa heat-shock protein in Vero cells. Heat-shock proteins (HSPs) are a set of polypeptides, whose synthesis represents a finely regulated response of prokaryotic and eukaryotic cells to adverse environmental conditions, among which heat-shock (Schlesinger et al., 1990). In eukaryotic cells, HSPs are present as multigene families, consisting of closely related protein isoforms, with members being expressed at low levels in unstressed cells (constitutive HSPs), and others expressed in response to a change in environmental conditions (inducible HSPs). It has been previously shown that cyclopentenone prostaglandins induce the synthesis of specific HSPs in several types of mammalian cells, in a non-stress situation (Santoro et al., 1989a; 1990; Amici et al., 1992a). In particular, in human erythroleukemic cells, it has been recently shown that, similarly to heat-shock, induction of a 70 kDa HSP (HSP70) by PGA_1 is mediated by heat shock factor (HSF) activation (Amici et al., 1992b).

In Vero cells, PGA_1 induces HSP70 synthesis dose-dependently and this effect is completely suppressed by actinomycin D. A possible role for HSP70 in virus replication has been suggested by different studies. Adenovirus infection of human cells increases HSP gene expression (Kao and Nevins, 1983), while infection of monkey or mouse cells with simian virus 40 or polyoma virus induces the synthesis of two host, heat-inducible 92 kDa and 72 kDa proteins (Khandjian and Turler, 1983). A possible role of HSPs in the arrest of virus growth and survival of the infected cells has been suggested in a human neuroblastoma model infected with HSV-2 (Yura et al., 1987). HSP70 has also been recently shown to be associated with the block of Sendai virus replication in monkey kidney cells, after treatment with PGA_1 (Amici and Santoro, 1991) or Δ^{12}-PGJ_2 (Amici et al., 1992a).

In the case of Sindbis virus, we have demonstrated that the antiviral activity of prostaglandins is mediated by a cellular product. The fact that actinomycin D completely prevented both the expression of HSP70 and the block of virus replication induced by PGA_1, suggests that this protein could be involved at some level in the block of Sindbis virus replication.

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