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Effect of combined αIFN and prostaglandin A_1 treatment on vesicular stomatitis virus replication and heat shock protein synthesis in epithelial cells

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Abstract

The antiviral activity of prostaglandin A (PGA) and interferons (IFNs) has been widely described. In the present report, we investigated the effect of combined αIFN and PGA_1 treatment on vesicular stomatitis virus (VSV) replication and on heat shock protein (HSP) induction in monkey epithelial cells. In uninfected cells, PGA_1 caused a dose-dependent induction of HSP70, HSP90 and HSP110, while αIFN did not affect HSP synthesis. Alpha-IFN suppressed VSV replication dose-dependently, even when cells were treated after virus infection. VSV protein synthesis was not affected by αIFN, indicating a block at the level of virus assembly or maturation. PGA_1 caused a dose-dependent inhibition of VSV replication, and suppressed VSV protein synthesis at concentrations which induced the synthesis of high levels of HSP70. The combined treatment with low doses of αIFN or PGA_1, which only moderately inhibited VSV replication when administered separately, was found to suppress VSV production by more than 95%, and resulted in a 3-fold increase of HSP70 synthesis as compared to PGA_1 alone. These results demonstrate a co-operative effect of PGA_1 and αIFN against VSV infection and suggest that αIFN can potentiate the cellular response to HSP induction in virus-infected cells.

Keywords: Glycosylation; Heat shock proteins; Interferon; Prostaglandins; Vesicular Stomatitis Virus

1. Introduction

Interferon (IFN) inhibits the multiplication of a wide variety of RNA and DNA viruses in animal cells. However, different steps in the virus replication cycle are affected by IFN depending on the virus species (Cantell and Schellekens, 1987; Gewert and Finter, 1992; Darnell et al., 1994). In the case of many RNA viruses, the major effect of IFN action appears to be the inhibition of the accumulation of viral proteins in infected cells, and IFN treatment has to be started several hours before virus infection for the antiviral activity to

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occur. In the case of vesicular stomatitis virus (VSV), interferon can inhibit virus growth at a variety of levels in its replication. These steps include penetration (Whitaker-Dowling et al., 1983), primary transcription (Marcus et al., 1971), cap methylation (De Ferra and Baglioni, 1981), protein synthesis (Baxt et al., 1977), and assembly (Maheshwari et al., 1980). The mechanism of IFN action also varies depending on the cell type (Thacore, 1978; Whitaker-Dowling et al., 1987).

Prostaglandins (PGs), a class of naturally occurring cyclic 20-carbon fatty acids, participate in the regulation of a variety of physiological and pathological processes, including the immune response, cell proliferation and differentiation, and virus replication (Garaci et al., 1987). The ability of prostaglandins of the A type (PGAs) to inhibit virus replication and prevent the establishment of persistent infections was first reported in 1980 (Santoro et al., 1980). It is now well established that PGs containing an α,β-unsaturated carbonyl group in the cyclopentane ring structure (cyclopentenone PGs, i.e. PGAs and PGJs) inhibit the replication of a wide variety of DNA and RNA viruses, including poxviruses (Santoro et al., 1982), herpes viruses (Yamamoto et al., 1987), orthomyxoviruses (Santoro et al., 1988), paramyxoviruses (Santoro et al., 1980), togaviruses (Mastromarino et al., 1993), picornaviruses (Ankel et al., 1985), retroviruses (D'Onofrio et al., 1990a; D'Onofrio et al., 1990b; Ankel et al., 1991) and rhabdoviruses (Santoro et al., 1983a; Bader and Ankel, 1990). The mechanism of antiviral activity appears to be complex, and it has been associated with the induction of heat shock protein (HSP) synthesis (Amici and Santoro, 1991; Amici et al., 1992a; Santoro, 1994). In the case of vesicular stomatitis virus, it has been shown that the cyclopentenone prostaglandin Δ^{12}-PGJ_2 blocks VSV replication at two separate levels in the early and late phase of the virus replication cycle (Pica et al., 1993). If applied early in infection, Δ^{12}-PGJ_2 selectively inhibits VSV protein synthesis and prevents the virus-induced shut-off of host cell protein synthesis, an effect associated with induction of HSP70 synthesis. On the other hand, when administered during a late phase of the virus replication cycle (6 h p.i.), Δ^{12}-PGJ_2 suppresses infectious virus production by altering G protein glycosylation (Pica et al., 1993).

In the present report, we have investigated the effect of combined human natural αIFN and PGA_1 treatment on VSV replication and on HSP induction in epithelial monkey kidney cells.

2. Materials and methods

2.1. Cell culture and virus infection

Epithelial monkey kidney cells (MA104 cell line) were grown in either T-25 Falcon flasks or in 24-well Linbro plates in RPMI 1640 culture medium (Gibco) supplemented with 10% fetal calf serum (FCS, Gibco) and antibiotics at 37°C in a 5% CO_2 atmosphere. Vesicular stomatitis virus, Indiana serotype (Orsay), was kindly provided by Dr. E. Rodriguez-Boulan (Cornell University, Medical College, New York, NY). Confluent MA104 monolayers were washed with phosphate-buffered saline (PBS) and infected with VSV, 3 plaque forming units (P.F.U/cell). After incubation for 1 h at 37°C, virus inocula were removed and monolayers were washed three times with PBS and incubated with 1 ml of RPMI 1640 containing 2% FCS, human alpha interferon (hαIFN) (Alfaferone, Alfa-Wassermann) and/or prostaglandin A_1 (PGA_1)(Cayman Chemical Co.). PGA_1 was stored as a 100% ethanolic solution (10 mg/ml) at -20°C and was diluted to the appropriate concentration immediately before use. Control media contained the same concentration of ethanol diluent, which was shown not to affect cell or virus metabolism. HαIFN was stored at $+4$°C and diluted in culture medium immediately before use.

2.2. Virus titration

For VSV titration, medium was collected 12 h or 24 h post-infection (p.i.) and, after removing cell debris by centrifugation at 2000 rpm for 15 min, titers were determined by cytopathic effect 50% (CPE 50%) assay on confluent monolayers of MA104 cells in 96-well tissue culture dishes (6

dilutions for each sample, 8 wells for each dilution), as previously described in detail (Rodriguez-Boulan, 1983). The dilution that gives 50% cytopathic effect was determined by the interpolating procedure of Reed and Muench (1938).

2.3. DNA and RNA synthesis

DNA and RNA synthesis were determined in confluent monolayers of uninfected or VSV-infected MA104 cells by labeling with [^{3}H]-thymidine or [^{3}H]-uridine respectively (10 μCi/ml/2 x 10^5 cells, 24 h pulse, starting after the 1h adsorption period). The radioactivity incorporated into acid-soluble and insoluble material was determined as previously described (Santoro et al., 1982). Briefly, cells were washed three times with PBS and 0.4 ml 5% TCA was added to each culture. After 1 h, the radioactivity in acid-soluble material was determined. Acid-insoluble radioactivity was measured after washing 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.

2.4. Protein labeling and SDS/PAGE

At different times after VSV infection, confluent cell monolayers were labeled with [^{35}S]-methionine (10 μCi/2 $\times$ 10^5 cells, unless otherwise specified) in methionine-free medium containing 5% dialysed FCS. Cells were usually incubated for 15–30 min in methionine-free medium before labeling. After labeling, cells were washed, lysed in lysis buffer [2% SDS (sodium dodecyl sulfate), 10% glycerol, 0.001% bromophenol Blue, 0.1M DTT, (dithiothreitol) 0.0625M Tris-HCl (pH 6.8)] and the radioactivity incorporated was determined as previously described (Santoro et al., 1989a). Samples containing an equal amount of radioactivity were analysed by SDS polyacrylamide gel electrophoresis (PAGE) in a vertical slab gel apparatus (3% stacking gel, 10% resolving gel, unless otherwise specified) using the buffer system described by Laemmli (1970). Gels were washed, fixed in 10% acetic acid, 10% TCA, 30% methanol, dried under vacuum and autoradiographed using Kodak films (Eastman Kodak). Densitometric analysis of the autoradiograms was performed on a BioRad model 620 CCD videodensitometer (BioRad Laboratories, Richmond, CA, U.S.A.) and the quantitative evaluation of proteins was determined using the BioRad 1-D Analyst software. HSP70 and virus protein synthesis were expressed as percentage of total protein synthesis. The molecular weights (M_rs) values of polypeptides were calculated by comparison with the following markers: myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (31 kDa) and lysozyme (14 kDa).

2.5. Immunoblot analysis

For immunoblot analysis, an equal amount of protein for each sample was separated by SDS/PAGE as described above, and blotted onto nitrocellulose using the technique described by Burnette (1981). After transfer, filters were incubated with an anti-72/73 kDa HSP monoclonal antibody (diluted 1:500) from HeLa cells (Amersham), which has been shown to be reactive against human and monkey HSP70, in TEN-Tween 20 buffer, (0.05M Tris-HCl (pH 7.4, 5 mM EDTA, 0.15 M NaCl, 0.05% Tween 20) and the bound antibody was detected by horseradish peroxidase-linked sheep anti-mouse antibody. M_rs were calculated using Bio-Rad low M_r markers. For quantitative determination of protein synthesis, after immunodetection, the individual bands were excised from the blots and the radioactivity incorporated was measured in a β-scintillation counter, as previously described (Amici and Santoro, 1991).

2.6. Statistical analysis

Statistical analysis was performed using the Student's t-test for unpaired data. Data were expressed as the mean $\pm$ S.E. and P values of < 0.05 were considered significant.

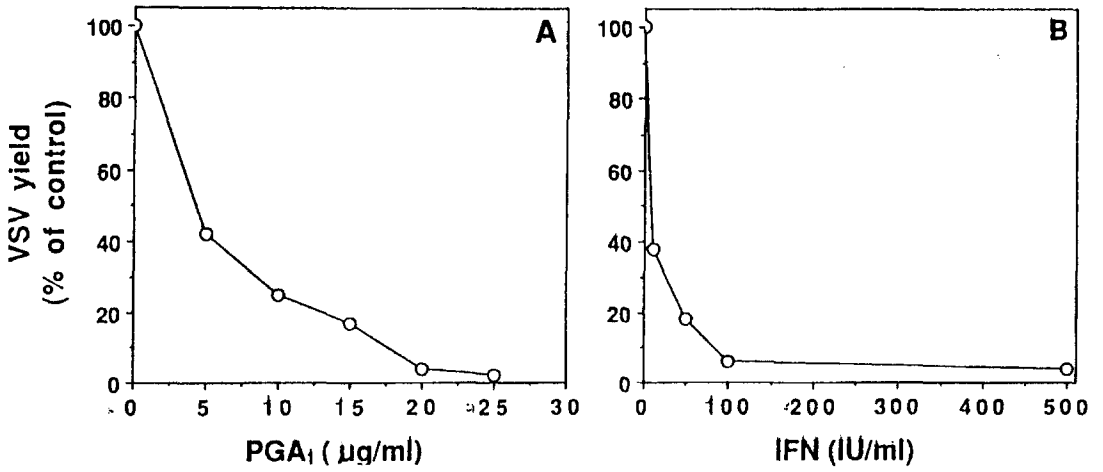

Fig. 1. Effect of PGA_1 or αIFN on VSV replication in MA104 cells. MA104 cells infected with VSV were treated with PGA_1 (A), αIFN (B) or ethanol diluent soon after the 1 h adsorption period. 24 h p.i. supernatants were collected and virus yields were determined by CPE 50% assay on confluent monolayers of MA104 cells. Virus yield of control = 3.4 ± 0.2 x 10^7 CPE 50% units/ml. Each point represents the mean of quadruplicate samples. Each experiment was repeated at least three times with the same results.

3. Results

3.1. Effect of PGA_1 and αIFN on VSV replication

In order to compare the antiviral activity of αIFN and PGA_1, confluent MA104 cell monolayers were infected with VSV for 1 h at 37°C. After removal of the viral inoculum, cells were treated with PGA_1 at different concentrations. The culture supernatants were collected 24 h after infection and the effect of PGA_1 on the viral output was determined by the CPE 50% assay. As shown in Fig. 1, PGA_1-treatment reduced virus yield in a dose-dependent manner, with a selective index of 32. Concentrations of PGA_1 between 20 and 25 µg/ml were found to suppress VSV production by more than 95% (Control = 3.4 ± 0.2 x 10^7; PGA_1 20 µg/ml = 3.7 ± 0.4 x 10^5 CPE 50% units/ml). An even more dramatic inhibition of virus production by PGA_1 was observed when virus titers were determined 10 h p.i. (Control = 4.5 ± 1.1 x 10^5; PGA_1 20 µg/ml = 4.47 ± 0.7 x 10^2 CPE 50% units/ml).

To test the effect of αIFN on VSV replication, cell monolayers were either pre-treated with αIFN for 16 h before virus infection or treated with different concentrations of the substance, starting after the adsorption period. As expected, even low concentrations of αIFN (10 IU/ml) were able to completely inhibit VSV replication and to protect the cells from the virus-induced cytopathic effect when MA104 cells were treated with αIFN 16 h before virus infection (data not shown). However, αIFN was able to suppress VSV replication in MA104 cells even when treatment was started immediately after virus infection. In this case, inhibition of virus replication was dose-dependent, and doses as low as 10 IU/ml were able to suppress virus yield by approximately 60% 24 h p.i. Concentrations of αIFN of 100–500 IU/ml suppressed VSV replication by more than 90% of control under the same conditions (Fig. 1B).

To study the effect of the combined treatment with αIFN and PGA_1 on VSV replication, MA104 cells were infected and, after the 1 h adsorption period, were treated with PGA_1, αIFN or both agents. Two types of schedules were performed to test the effect of treatment with low doses of PGA_1 (5 µg/ml) and αIFN (10 IU/ml), which partially inhibited VSV replication, or with high doses of PGA_1 (20 µg/ml) and αIFN (500 IU/ml), which suppressed virus yield by more than 90%, even when administered separately.

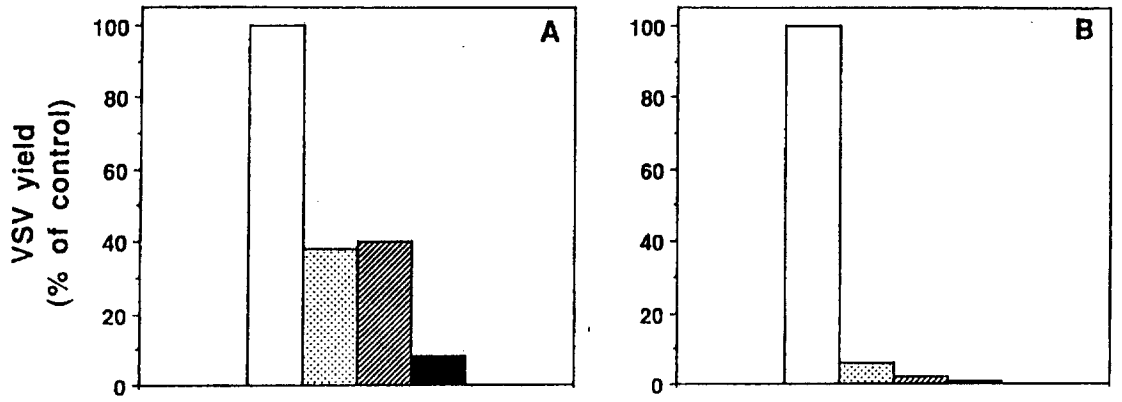

Fig. 2. Effects of $PGA_1/\alpha IFN$ combined treatment on VSV replication in MA104 cells. VSV-infected MA104 cells were treated soon after the 1 h adsorption period with the following concentrations of each antiviral agent: (A) = PGA_1 (5 μg/ml); αIFN (10 IU/ml); (B) = PGA_1 (20 μg/ml); αIFN (500 IU/ml), or with ethanol diluent. Virus yield of control = 3.63 $\pm$ 0.32 x 10^7 CPE 50% units/ml (panel A); 2.38 $\pm$ 0.2 x 10^7 CPE 50% units/ml (panel B). Data represent the mean of quadruplicate samples. In experiments shown in panels A and B, P values were < 0.05 for PGA_1-treated and αIFN-treated cells in relation to the untreated control, and for $PGA_1 + \alpha$IFN-treated cells in relation to cells treated with PGA_1 or αIFN alone. Each experiment was repeated at least three times with the same results. Ethanol (□), αIFN (▤), PGA_1 (▨), αIFN + PGA_1 (■).

Fig. 2 shows that the combined treatment with PGA_1 and αIFN was more effective than either of the two agents by itself. The enhancement of the antiviral effect was more evident when low doses of PGA_1 and αIFN were used (Fig. 2A).

3.2. Effect of PGA_1 and αIFN on nucleic acid synthesis in uninfected and VSV-infected MA104 cells

To study the effect of the combined treatment with low doses of PGA_1 and αIFN on nucleic acid synthesis in uninfected and VSV-infected cells, MA104 cell monolayers were treated with PGA_1 (5 μg/ml), αIFN (10 IU/ml) or both soon after infection and then labeled with [^{3}H]-thymidine or [^{3}H]-uridine for the following 24 h. Mock-infected cells were treated identically. The uptake of the radiolabeled precursors by the cells and their incorporation into DNA or RNA were determined as described in the Methods section. In uninfected cells PGA_1, at the dose tested, had no effect on [^{3}H]-thymidine and [^{3}H]-uridine uptake by the cells (Fig. 3A and A′), or on DNA and RNA synthesis (Fig. 3C and C′). Alpha-IFN only slightly decreased [^{3}H]-thymidine uptake and DNA synthesis (Fig. 3A and C) and it inhibited [^{3}H]-uridine uptake and RNA synthesis by approximately 25% (Fig. 3A′ and C′). The combined αIFN-PGA_1 treatment caused a slight decrease in [^{3}H]-thymidine or [^{3}H]-uridine uptake as well as DNA or RNA synthesis, which was not significantly different from the effect obtained after treatment with αIFN alone ($P > 0.05$), indicating no additional toxic effect (Fig. 3A, A′, C and C′). In VSV-infected cells, while PGA_1 had no effect, αIFN treatment caused an increase in [^{3}H]-thymidine uptake and incorporation into DNA (Fig. 3B and D), but had no effect on [^{3}H]-uridine uptake and RNA synthesis (Fig. 3B′ and D′). Again the combined treatment with PGA_1 and αIFN had the same effect on [^{3}H]-thymidine uptake and DNA synthesis as treatment with αIFN alone (Fig. 3B and D). In a parallel experiment, the effect of higher doses of PGA_1 (20 μg/ml), αIFN (500 IU/ml) or both on nucleic acid synthesis in uninfected and VSV-infected MA104 cells was determined. In uninfected cells, neither PGA_1 nor αIFN inhibited significantly DNA or RNA synthesis; the combined PGA_1/αIFN treatment caused a moderate (~35%) inhibition of DNA synthesis, while it had no effect on RNA synthesis (data not shown). In VSV-infected cells, αIFN had no effect on RNA or DNA synthesis, while

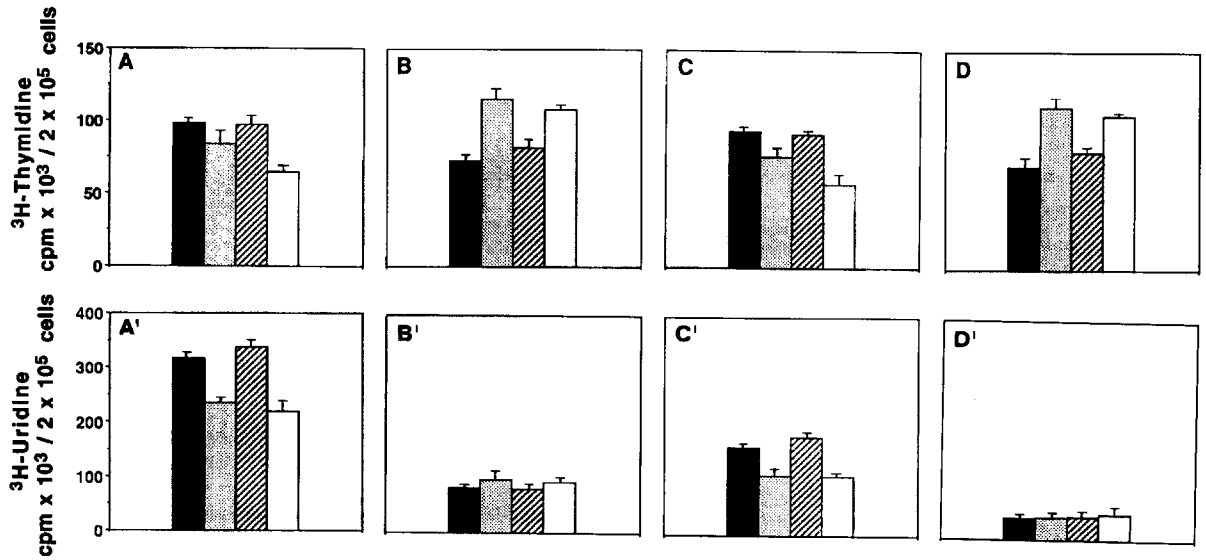

Fig. 3. Effect of PGA_1 and /or αIFN treatment on DNA and RNA synthesis in uninfected and VSV-infected MA104 cells. DNA and RNA synthesis were measured in confluent monolayers of uninfected (A, A', C, C') and VSV-infected (B, B', D, D') MA104 cells treated with PGA_1 (5 μg/ml), αIFN (10 IU/ml) or both soon after the 1 h adsorption period, and labeled with [^{3}H]-thymidine or [^{3}H]-uridine respectively. The radioactivity incorporated into acid-soluble (A, B, A', B') and acid-insoluble (C, D, C', D') material was determined 24 h p.i. Data represent the mean ± S.D. of duplicate samples. Control (■), αIFN (▩), PGA_1 (▨), αIFN + PGA_1 (□).

PGA_1-treatment alone or in association with αIFN resulted in an increase of both DNA and RNA synthesis in infected cells ([^{3}H]-thymidine incorporation: Control = 4.3 ± 0.4 x 10^5; αIFN = 3.7 ± 0.6 x 10^5; PGA_1 = 6.4 ± 0.9 x 10^5; PGA_1 + αIFN = 6.8 ± 0.8 x 10^5 c.p.m./10^6 cells; [^{3}H]-uridine incorporation: Control = 1.91 ± 0.06 x 10^6; αIFN = 1.78 ± 0.05 x 10^6; PGA_1 = 2.91 ± 0.6 x 10^6; PGA_1 + αIFN = 2.86 ± 0.1 x 10^6 c.p.m./10^6 cells). The increase in DNA and RNA synthesis in PGA_1-treated infected cells is consistent with the cytoprotective effect of PGA_1, which, at this concentration, is able to prevent the VSV-induced shut-off of host cell metabolism, as described below.

3.3. Effect of PGA_1 and αIFN-treatment on cellular and viral protein synthesis

In order to study the effect of PGA_1, αIFN or PGA_1-αIFN combined treatment on cellular and VSV protein synthesis, MA104 cell monolayers were infected with VSV and treated with 5 μg/ml PGA_1, 10 IU/ml αIFN or both, soon after the 1 h adsorption period. Cells were labeled with [^{35}S]-methionine 6 h after the beginning of treatment for the following 4 h. Mock-infected cells were treated identically. After determining the radioactivity incorporated into the TCA-insoluble material, samples containing the same amount of radioactivity were processed for SDS-PAGE analysis and autoradiography. In a parallel experiment, unlabeled cells were treated identically and samples containing the same amount of proteins were processed for immunoblot analysis, using anti-HSP70 monoclonal antibodies. In uninfected cells, αIFN-treatment had no apparent effect on protein synthesis at this time, while PGA_1 only slightly inhibited [^{35}S]-methionine incorporation into proteins and induced the synthesis of a 72-KDa molecular weight protein (Fig. 4A), which was identified as a 70-kDa heat shock protein (HSP70) by immunoblot analysis (Fig. 4B). The synthesis of the 90-kDa heat shock protein (HSP90) was slightly increased by PGA_1. The electrophoretic pattern of cells treated with both

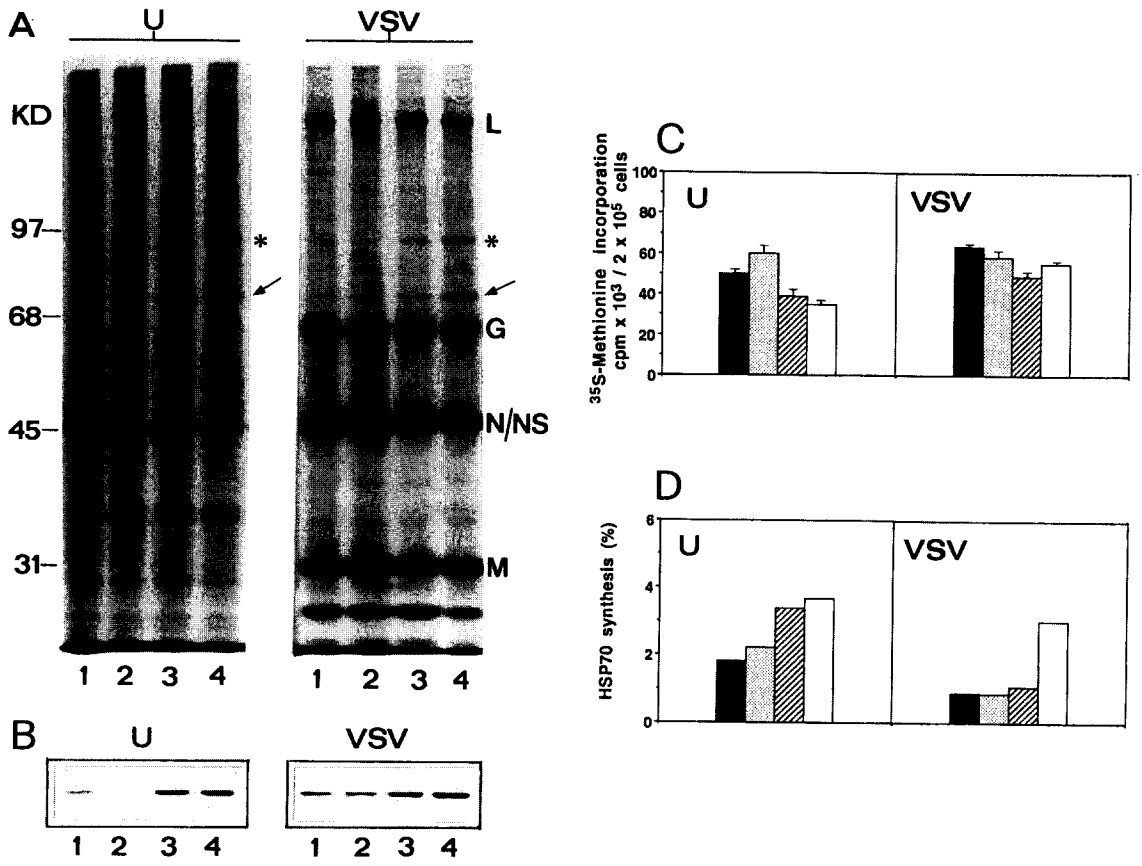

Fig. 4. Effect of low doses of PGA_1 and/or αIFN on the synthesis of cellular and viral proteins in MA104 cells. Uninfected (U) or VSV-infected (VSV) MA104 cells were treated with αIFN (10 IU/ml, lane 2), PGA_1 (5 μg/ml, lane 3), PGA_1 + αIFN (lane 4) or control diluent (lane 1) soon after virus infection, and labeled with [^{35}S]-methionine (10 μCi/ml, 4 h pulse starting 6 h p.i.). (A) SDS-PAGE analysis of radiolabeled polypeptides. HSP70 is indicated by arrow. HSP90 is indicated by the asterisk. (B) Identification of HSP70 in samples described in (A) by immunoblot analysis using anti-HSP70 monoclonal antibodies. (C) Incorporation of [^{35}S]-methionine into TCA-insoluble material of samples described in (A). P values were < 0.05 for PGA_1-treated and PGA_1 + αIFN-treated uninfected cells in relation to αIFN-treated uninfected cells. (D) HSP70 synthesis was quantified by densitometric analysis, and expressed as percent of total protein synthesis. Control (■), αIFN (▩), PGA_1 (▨), αIFN + PGA_1 (□).

PGA_1 and αIFN was apparently identical to the one from cells treated with PGA_1 alone, and densitometric analysis revealed that the amount of HSP70 synthesized in cells treated with PGA_1 alone or with PGA_1-αIFN was essentially equivalent (Fig. 4A and D). Alpha-IFN alone did not significantly induce HSP70 synthesis (Fig. 4A and D).

In VSV-infected cells, cellular protein synthesis was almost completely shut off 10 h after infection. Treatment with αIFN (10 IU/ml) or PGA_1 (5 μg/ml), even though it inhibited VSV production by approximately 60%, had no apparent effect on virus protein synthesis at these concentrations (Fig. 4A), suggesting that both drugs were affecting a late step in the virus replication cycle. Combined αIFN-PGA_1 treatment, which was able to suppress VSV production by more than 95%, did not significantly affect virus protein synthesis (Fig. 4A). Immunoblot analysis revealed

that the level of HSP70 was slightly higher in VSV-infected as compared to uninfected cells. A moderate increase in the level of HSP70 was also noted in PGA_1-treated cells as compared to untreated cells by western blot. Densitometric analysis of autoradiograms shown in Fig. 4A indicated that, in VSV-infected cells, while HSP70 synthesis was not significantly induced by treatment with either αIFN or PGA_1 alone at these concentrations, it was increased by approximately 3 times when PGA_1 and αIFN were administered simultaneously (Fig. 4A and D), indicating that in virus-infected cells αIFN potentiates the cellular response to PGA_1.

We then studied the effect of high concentrations of PGA_1 (20 μg/ml) or αIFN (500 IU/ml) which were both able to inhibit VSV production by more than 90%, on cellular and viral protein synthesis. MA104 cell monolayers were infected with VSV, treated with PGA_1, αIFN or both soon after the 1 h adsorption period, and labeled with [^{35}S]-methionine as described above. Mock-infected cells were treated identically. Under the conditions tested, in uninfected cells both αIFN and PGA_1 decreased total protein synthesis by approximately 30% as compared to control (Fig. 5C). Samples containing an equal amount of radioactivity were processed for SDS-PAGE analysis and autoradiography. In a parallel experiment, cells were treated identically and samples containing the same amount of protein were processed for immunoblot analysis using anti-HSP70 monoclonal antibodies. PGA_1 strongly induced the synthesis of 70 kDa, 90 kDa and 110 kDa heat shock proteins, while αIFN did not induce HSP synthesis (Fig. 5A). The combined αIFN-PGA_1 treatment did not further decrease cell protein synthesis, and the electrophoretic pattern of the proteins synthesized appeared to be similar to the one from the cells treated with PGA_1 alone. In VSV-infected cells, αIFN, even at the high dose, did not visibly suppress VSV protein synthesis, suggesting that αIFN is blocking virus protein maturation or assembly. On the other hand, PGA_1 at the effective dose (20 μg/ml) was found to dramatically suppress virus protein synthesis, and to protect MA104 cells from the virus-induced shut-off of cellular protein synthesis (Fig. 5A and D). The effect of αIFN-PGA_1 treatment on cell and virus protein synthesis appeared to be similar to the one of PGA_1 alone. At the high concentration, PGA_1 was found to dramatically increase HSP70 synthesis even in VSV-infected cells, and the simultaneous presence of αIFN could not further increase HSP70 levels (Fig. 5A, B and E).

4. Discussion

Vesicular stomatitis virus, a negative strand RNA virus, has been widely used in studies investigating the mechanism of action of antiviral drugs and of IFN in particular. Following the penetration into the cells, VSV genome is first transcribed to form mRNAs which are then translated into viral proteins. This primary RNA transcription and the consequent translation of primary transcripts prepare for the replication of the viral genome, which is necessary for the amplification of transcription and translation leading to a massive accumulation of viral proteins. When epithelial monkey MA104 cells are infected with VSV (3 P.F.U./cell), virus protein synthesis starts approximately 3–4 h after infection, and by 6 h p.i. cellular protein synthesis is shut off and only virus proteins are synthesized. VSV cytopathic effect, which causes cell rounding and detachment from substrata, is evident 8–10 h after infection, and 100% cell death is observed after 24 h (Pica et al., 1993).

Cyclopentenone prostaglandins inhibit VSV replication at two separate levels related to two distinct events in the early and late phase of the virus cycle (Pica et al., 1993). It has been shown that treatment of MA104 cells with the cyclopentenone prostaglandin Δ^{12}-PGJ_2, if administered during a late phase of VSV replication (6 h after infection with 3 P.F.U./cell) after the amplification of transcription and translation, suppresses infectious virus production by altering G glycoprotein maturation (Pica et al., 1993). PGA_1 has also been shown to inhibit G protein maturation in VSV-infected murine L fibroblasts (Santoro et al., 1983a). However, when treatment with Δ^{12}-PGJ_2 is started soon after the 1 h adsorption

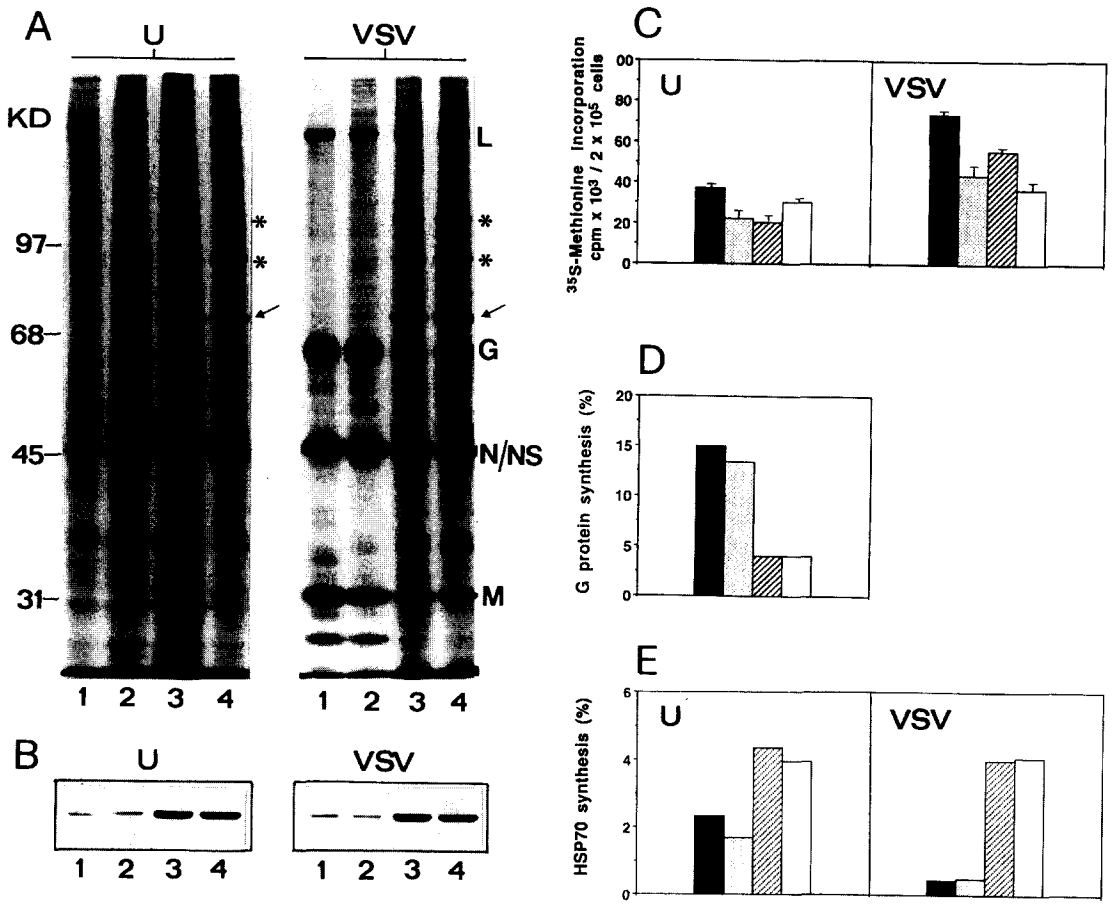

Fig. 5. Effect of active doses of PGA_1 and/or αIFN on the synthesis of cellular and viral proteins in MA104 cells. Uninfected (U) or VSV-infected (VSV) MA104 cells were treated with αIFN (500 IU/ml, lane 2), PGA_1 (20 μg/ml, lane 3), PGA_1 + αIFN (lane 4) or control diluent (lane 1) soon after virus infection and labeled with [^{35}S]-methionine (10 μCi/ml, 4 h pulse starting 6 h p.i.). (A) SDS-PAGE analysis of radiolabeled polypeptides. HSP70 is indicated by arrow. HSP90 and HSP110 are indicated by asterisks. (B) Identification of HSP70 in samples described in (A) by immunoblot analysis using anti-HSP70 monoclonal antibodies. (C) Incorporation of [^{35}S]-methionine into TCA-insoluble material of samples described in (A). P-values were < 0.05 for PGA_1-treated, αIFN-treated, and PGA_1 + αIFN-treated VSV-infected cells in relation to untreated VSV-infected cells. VSV G glycoprotein (D) and HSP70 (E) synthesis were quantified by densitometric analysis, and expressed as percent of total protein synthesis. Control (■), αIFN (▩), PGA_1 (▨), αIFN + PGA_1 (□).

period, it results in a dramatic block of VSV protein synthesis and in a partial protection of the host cell from the virus-induced shut off of cellular protein synthesis. This effect has been associated with the induction of the synthesis of a 70-kDa heat shock protein (HSP70) in MA104 cells (Pica et al., 1993).

Heat shock proteins are a group of polypeptides the synthesis of which is induced in prokaryotic as well as in eukaryotic cells by heat shock or other environmental stresses; they can be divided into five families with M_r of 15–30 kDa (low M_r HSP), 60 kDa (HSP60), 70 kDa (HSP70), 90 kDa (HSP90) and 100–110 kDa (HSP110), respectively. In eukaryotic cells, HSPs are generally present as multigene families, consisting of closely related protein isoforms, with members being expressed in unstressed cells (constitutive HSPs)

and others following heat treatment (inducible HSPs) (Lindquist and Craig, 1988; Schlesinger, 1990; Morimoto et al., 1992). The structure of HSPs, and HSP70 in particular, has been highly conserved throughout evolution from bacteria to man (Lindquist and Craig, 1988). In eukaryotic cells, HSP70 proteins are needed for protein folding, assembly and intracellular transport, and can bind to and dissociate protein complexes in the presence of ATP (Morimoto, 1993).

Prostaglandin A_1 induces HSP70 gene transcription by cycloheximide-sensitive activation of heat shock transcription factor (HSF) (Santoro et al., 1989b; Amici et al., 1992b). Induction of HSP70 gene expression by PGA_1 has now been described in a large variety of monkey, canine, porcine and human cell lines, as well as in human peripheral blood lymphocytes and macrophages, and in primary cells derived from cord blood (reviewed in Santoro et al., 1990). Induction of HSP70 by PGA_1 has been associated with the antiviral activity of this compound, and we have recently shown that HSP70 interferes with virus RNA translation in cells infected with Sendai virus (Amici et al., 1994; Santoro, 1994).

In the present report, we have investigated the effect of PGA_1, αIFN or the combined αIFN-PGA_1 treatment on VSV replication and on HSP induction in epithelial monkey kidney cells. As previously shown in other cell lines (Santoro et al., 1990), in uninfected cells PGA_1 caused a dose-dependent induction of HSP70, HSP90 and HSP110. Under the conditions examined, αIFN did not affect HSP synthesis; moreover, αIFN did not modify the amount of HSP70 induced by PGA_1 when used in combined treatment in uninfected MA104 cells.

In VSV-infected cells PGA_1 treatment, started soon after the 1 h adsorption period, caused a dose-dependent inhibition of VSV replication. At the concentration of 5 μg/ml, which inhibited virus production by approximately 60%, VSV protein synthesis did not appear to be greatly affected, indicating an effect on a late stage of virus replication. Interestingly, at this concentration, PGA_1 was not able to induce HSP70 synthesis in VSV-infected cells, indicating that VSV infection can interfere with HSP70 induction depending on PGA_1 concentration. However, at the concentration of 20 μg/ml, which inhibited VSV production by more than 95%, PGA_1 caused a dramatic suppression of VSV protein synthesis and it partially protected the host cell from the virus-induced shut off of cellular protein synthesis. As it was previously shown for Δ^{12}-PGJ_2, PGA_1 at this concentration was able to induce HSP70 synthesis in VSV-infected cells at the same level as in control uninfected cells. These results indicate that the selective inhibition of virus protein synthesis by PGA_1 is, also in this case, associated with the accumulation of high intracellular levels of HSP70.

Under the conditions examined, αIFN suppressed VSV replication in a dose-dependent manner. Virus protein synthesis was not affected by αIFN when cells were treated after virus infection. This is consistent with a block at the level of virus assembly or maturation, as previously shown in different cell lines (Maheshwari et al., 1980). Alpha-IFN did not induce HSP70 synthesis also in VSV-infected cells.

While treatment with low dose of αIFN (10 IU/ml) or PGA_1 (5 μg/ml) inhibited VSV production by approximately 60% when administered separately, the combined αIFN/PGA_1 treatment was found to suppress VSV production by more than 95%, and resulted in a 3-fold increase of HSP70 synthesis, as compared to treatment with PGA_1 alone. Since it has been previously shown that IFN is able to stimulate PGE and PGA synthesis in virus-infected cells (Santoro et al., 1983b), this effect could be due to an increase of endogenous prostaglandin synthesis in IFN-treated cells.

The observation that the combined treatment with low doses of PGA_1 and αIFN, which are not toxic to uninfected cells and only moderately influence cellular nucleic acid or protein synthesis, potentiates the antiviral activity as compared to either of the two agents alone, opens new interesting perspectives in antiviral chemotherapy. However, it is not clear at the moment whether this co-operative effect could be related to the stimulation of PGA_1-induced HSP70 synthesis by αIFN in these cells. Treatment with αIFN, which is not able to induce HSP synthesis in mammalian cells,

has been shown to potentiate the transcription and translation of HSP mRNA after a mild heat shock (39.5°–40.5°C) in human cells (Chang et al., 1991; Vokes et al., 1989). In murine cells, interferon pretreatment is able to lower the threshold for maximal heat-shock response, and to stimulate the accumulation of HSPs (Morange et al., 1986). In mouse L cells, it has been shown that the regulation of HSP expression by IFN takes place at two levels, by increasing the rate of HSP gene transcription and by increasing the stability of mRNAs coding for HSPs (Dubois et al., 1988). The fact that also in virus-infected MA104 cells, αIFN increases the level of HSP70 synthesis induced by PGA_1 at low concentration reinforces the concept that IFN can enhance the effect of different types of HSP inducers. In our model, it remains to be established whether the increase in HSP70 synthesis by αIFN co-treatment is due to a specific activity of IFN, or is the consequence of a generalized cytoprotective effect of interferon in virus-infected cells.

In view of the cytoprotective role of heat shock proteins during the pathogenesis of several disease states (Amici et al., 1994; Iaattela et al., 1992; Williams et al., 1993), the ability of interferon to enhance HSP70 synthesis under conditions of mild stimulation could then potentiate HSP70 cytoprotective role in different pathological states, including fever and acute virus infection.

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