Suppression of virus replication by prostaglandin A is associated with heat shock protein synthesis

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The antiviral action of cyclopentenone prostaglandins (PGs) is generally associated with alterations in the synthesis and/or maturation of specific virus proteins. In particular, inhibition of Sendai virus (SV) replication in African green monkey kidney cells by PGA1 has been shown to be a cell-mediated event, due to alterations in SV protein glycosylation and accompanied by the induction of a cellular polypeptide of Mr 74K. In this report we identify this protein as a heat shock protein (HSP) related to the major 70K HSP group (HSP70). Induction of HSP70 synthesis by PGA1 was found to be dose-dependent, and an accumulation of HSP70 comparable to that occurring after heat shock could be obtained at concentrations of PGA1 that did not inhibit macromolecular synthesis in uninfected cells, but caused a dramatic block of virus replication in SV-infected cells. Induction of HSP70 by PGA1 occurred at the transcriptional level and was not affected by SV infection. HSP70 synthesis was evident between 2 and 3 h after PGA1 treatment, maximal at 12 h and went back to control levels by 26 h after the addition of PGA1, thus preceding virus protein synthesis. Finally, of several PGs tested, only those which possess antiviral activity induced the synthesis of HSP70. These results, together with the observation that suppression of HSP70 synthesis by actinomycin D also abolishes the PGA1-induced alteration of SV glycoproteins, suggest that HSP70 could play a role in the block of virus replication by cyclopentenone PGs.

Introduction

The first observation that prostaglandins (PGs) of the A type (PGAs) can potently inhibit the replication of a paramyxovirus [Sendai virus (SV)] and prevent the establishment of a persistent infection in cultured cells was made in 1980 (Santoro et al., 1980). Since that time the antiviral activity of PGAs and, more recently, PGJs has been demonstrated for a wide variety of DNA and RNA viruses, including orthomyxoviruses (Santoro et al., 1988), rhabdoviruses (Santoro et al., 1983), picornaviruses (Ankel et al., 1985), togaviruses (Mastromarino et al., 1990), poxviruses (Santoro et al., 1982a), herpesviruses (Santoro, 1987; Yamamoto et al., 1989) and retroviruses (D’Onofrio et al., 1990a, b), in different types of mammalian cells. The in vivo antiviral activity of a long-acting synthetic analogue of PGA2 [16,16-dimethyl-PGA2 methyl ester (di-M-PGA2)] in a mouse model infected with influenza A virus has also been shown recently (Santoro et al., 1988). The antiviral activity appears to be related to the presence of an α,β-unsaturated carbonyl group in the cyclopentane ring of the molecule, because it is specific for PGs of the A and J type (cyclopentenone PGs); PGs of the B, E and F series, prostacyclin, 6-keto PGF1α and thromboxane B2 do not suppress virus replication (reviewed in Santoro, 1987).

PGAs suppress virus replication dose-dependently and greater than 95% inhibition of virus production can be obtained at doses which do not affect DNA, RNA or protein synthesis in uninfected cells. The target for PGA action appears to be a late event in the virus replication cycle, and, in the majority of virus–cell models studied, PGA treatment causes an alteration in the synthesis and/or maturation of one or more virus late proteins (reviewed in Santoro, 1987).

We have shown previously that PGA1 can almost totally suppress SV replication in an African green monkey kidney (AGMK) cell line at concentrations which do not inhibit protein synthesis and which induce the synthesis of a 74K polypeptide, p74, in both uninfected and virus-infected cells (Santoro et al., 1981, 1982b). Recently we have shown that inhibition of SV replication in AGMK cells by PGA1 is due to alterations in SV protein glycosylation and that this event is cell-mediated (Santoro et al., 1989a). Since p74 is the only host polypeptide found to be induced by PGA1 in these
cells and its synthesis appears to be associated with the PGA1-induced alterations in virus glycoproteins, identification of this protein appeared to be an important step in the understanding of the mechanism by which PGs block virus replication. On the basis of the Mr and other biochemical characteristics, which are similar to those of the major mammalian 72K to 73K heat shock protein (HSP) (Burdon, 1982), we hypothesized that p74 could belong to this group of proteins (Santoro, 1987).

In this report we identify this protein as an HSP related to the major HSP70 group, describe its metabolism and discuss its possible role in the inhibition of SV replication.

Methods

Cell culture. The establishment and culture of AGMK 37RC cells have been described in detail (Santoro et al., 1981). Briefly, cells were grown in either T-25 Falcon flasks or in 24-well Linbro plates in RPMI 1640 medium, supplemented with 5% foetal calf serum (FCS; Gibco) and antibiotics, at 37 °C in a 5% CO2 atmosphere. PGA1, PGA2, PGB2, PGD2, PGE1, PGE2, PGF1, 6-keto PGF1, thromboxane B2 (Sigma), PGJ2, and d15-PGJ2 (Cayman) were stored as 100~ethanolic solutions (2 mg/ml) and tested at a concentration of 4 μg/ml unless otherwise specified. Control medium contained the same concentration of ethanol (0.02%), which did not affect cell metabolism or virus replication. DI-M-PGA1 was a generous gift from Dr J. Pike, the Upjohn Co., U.S.A.

Virus infection, titration and purification. Preparation of SV by allantoic inoculation of embryonated eggs has been described previously (Santoro et al., 1989a). For virus infection, confluent cell monolayers were washed with PBS and virus samples were added (0-5 ml for Linbro wells and 2 ml for the flasks). 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 (for Linbro wells) or 5 ml (for the flasks) of RPMI 1640 containing 2% FCS. Virus production was determined by measuring the number of haemagglutinin units (HAU) present in the medium of infected monolayers of 37RC cells at different times post-infection (p.i.). Haemagglutinin titrations were done according to standard procedures using human type O Rh+ red blood cells (Santoro et al., 1989a). For virus purification, 37RC cells grown in 100 mm Petri dishes were infected with SV and labelled with [35S]methionine (5 μCi/ml) in RPMI 1640 containing 2% FCS soon after infection. Culture supernatants were collected 48 h p.i. and clarified at 6000 g for 20 min. Supernatants were diluted in PBS and centrifuged at 12000 g for 2 h in a 45Ti rotor (Beckman), according to the method described by Hsu et al. (1979). The radioactivity of purified virus from untreated cells averaged 4 × 106 c.p.m./mg protein.

PAGE. At different times after SV infection, confluent cell monolayers were labelled with [35S]methionine (5 μCi/2 × 106 cells, unless otherwise specified) in methionine-free medium containing 5% dialysed FCS. Cells were usually preincubated for 15 min in methionine-free medium. After labelling, cells were washed, lysed in lysis buffer (2% SDS, 10% glycerol, 0.001% bromphenol blue, 0.1 M DTT, 0.0625 M-Tris–HCl pH 6.8) and the radioactivity incorporated was determined as described previously (Santoro et al., 1982a). Samples were analysed by SDS–PAGE in a vertical slab gel apparatus (3% stacking gel, 7.5% resolving gel, unless otherwise specified) 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). The Mr of polypeptides were calculated by comparison with the following markers: myosin (200K), β-galactosidase (116K), phosphorylase b (97K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (31K) and lysozyme (14K).

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-72/73K HSP monoclonal antibody (Mab) (diluted 1:500) from HeLa cells (Amersham), which has been shown to be reactive against human and monkey HSP72, 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). Mr were calculated using Bio-Rad low Mr markers.

Statistical analyses. Statistical analyses were performed using the Student’s t-test for unpaired data. Data were expressed as the mean ± S.E.M.; P values of <0.05 were considered significant. For haemagglutinin titres, an S.E.M. of 0 indicates that HAU titres were identical for each pair of samples.

Results

PGA1 treatment blocks SV budding from 37RC cells

PGA1 has been shown previously to inhibit SV replication by altering virus protein glycosylation and inhibiting the incorporation of viral glycoprotein haemagglutinin–neuraminidase (HN) into the host cell membrane (Santoro et al., 1989a). To determine whether these events could cause a total block of virus maturation and budding from infected cells, or production of defective virus particles lacking the HN glycoprotein, confluent monolayers of 37RC cells were infected with SV and, after the 1 h infection period, were treated with PGA1 (4 μg/ml) or control diluent and labelled with [35S]methionine (5 μCi/ml) for the following 48 h. Uninfected cells were treated identically. After 48 h, supernatants from infected or mock-infected cells were collected, and the presence of virus particles was determined by SDS–PAGE protein separation and autoradiography after virus purification (Fig. 1). Virus replication was assayed by measuring the haemagglutination activity in the medium of unlabelled SV-infected cells treated identically. PGA1 treatment had not inhibited protein synthesis in uninfected cells after 48 h, whereas it moderately suppressed it in SV-infected cells (Fig. 1a). SDS–PAGE of [35S]methionine-labelled proteins after virus purification revealed a dramatic decrease in the amount of all virus proteins after PGA1 treatment (Fig. 1b,c), demonstrating that the PGA1-induced alteration in virus protein glycosylation described previously results in the block of virus maturation and budding from infected cells. Analogous results were obtained in a parallel experiment in which virus particles from the superna-
Identification of the PGA₁-induced p74 polypeptide as an HSP

The block of SV protein glycosylation by PGA₁ is accompanied by the induction of the synthesis of a 74K cellular protein (p74). To investigate whether the PGA₁-induced p74 could be an HSP, confluent monolayers of 37RC cells were infected with SV and, 1 h after infection, virus inocula were removed and culture medium containing PGA₁ (4 μg/ml) or control diluent was added; uninfected cells were treated identically. Cells were labelled with [³⁵S]methionine (10 μCi/well) for 2 h at different times after SV infection and, after determining the radioactivity incorporated into TCA-insoluble material, cell extracts were separated by SDS-PAGE and processed for autoradiography. Fig. 2(a) shows the accumulation of a 74K protein in SV-infected or uninfected, PGA₁-treated cells 20 h after treatment. Induction of p74 synthesis appeared to be similar in both SV-infected cells and uninfected cells. In a parallel experiment, confluent monolayers of uninfected or SV-infected 37RC cells treated with 4 μg/ml PGA₁ or control diluent were lysed in lysis buffer 24 h p.i. An equal amount of protein from different samples was separated by SDS-PAGE and processed for immunoblot analysis using anti-human 72/73K HSP Mabs. Immunoblot analysis revealed the presence of a unique band of approximate Mr 74K in uninfected or SV-infected, PGA₁-treated cells (Fig. 2b), identifying p74 as an HSP70.

To investigate whether PGA₁ was acting on the transcription or translation of HSP70 mRNA, a similar experiment was performed in the presence of actinomycin D (2 μg/ml), which suppresses cellular but not viral RNA synthesis (SV RNA polymerase being resistant to

Fig. 1. Effect of PGA₁ treatment on SV production by 37RC cells. (a) Uninfected or SV-infected 37RC cells were treated with PGA₁ (4 μg/ml) or ethanol control and labelled with [³⁵S]methionine soon after infection. PGA₁ treatment did not alter protein synthesis, as measured by [³⁵S]methionine incorporation into acid-insoluble material, in uninfected cells and slightly decreased it in SV-infected cells 48 h p.i. Data represent the mean ± s.d. of duplicate samples. □, Uninfected; ■, uninfected, PGA₁-treated; ■, SV-infected; □, SV-infected, PGA₁-treated cells. (b) [³⁵S]Methionine incorporation into proteins obtained from culture medium of uninfected or SV-infected cells (48 h p.i.) after virus purification. (c) SDS–PAGE of [³⁵S]methionine-labelled proteins from purified virus collected from the culture medium 48 h p.i. No virus protein was detected in the supernatant of PGA₁-treated, SV-infected cells. Virus yield (HAU/2 × 10⁵ cells) was measured in a parallel experiment in the medium of unlabelled SV-infected cells treated identically. Lanes 1 and 2, uninfected cells; lanes 3 and 4, SV-infected cells. Samples in lanes 1 and 3 were treated with ethanol control, lanes 2 and 4 with PGA₁.
Fig. 2. Identification of p74 as an HSP70. (a) SDS-PAGE analysis of [35S]methionine-labelled polypeptides from uninfected (U) or SV-infected (SV) 37RC cells, treated with PGA1 (4 μg/ml) (lanes 2 and 4) or control diluent (lanes 1 and 3) soon after virus infection. PGA1 treatment induced p74 synthesis to the same extent in both uninfected and SV-infected cells. (b) Proteins from samples treated as in (a) were separated by SDS-PAGE and processed for immunoblot analysis using anti-72/73K HSP MAbs which revealed the presence of a unique 74K protein, the synthesis of which was several-fold greater than constitutive levels after PGA1 treatment. (c) 37RC cells were treated with PGA1 (4 μg/ml) or control diluent in the presence (lanes 3 and 4) or the absence (lanes 1 and 2) of actinomycin D (2 μg/ml). After 24 h cells were lysed and proteins processed for immunoblot analysis using the anti-HSP70 MAb. Actinomycin D completely prevented HSP70 induction by PGA1. (d) An equal amount of protein from 37RC cells, treated for 24 h with different concentrations of PGA1 (lanes 1 to 5, 0, 1, 2, 4 and 8 μg/ml PGA1), was separated by SDS-PAGE and processed for immunoblot analysis. HSP70 accumulation increased dose-dependently.

actinomycin D) (Santoro et al., 1981). Fig. 2(c) shows that treatment with actinomycin D totally prevented HSP70 induction by PGA1, indicating an effect on HSP70 mRNA transcription.

Since PGA1 inhibits SV replication in a dose-dependent fashion (Santoro et al., 1980), we investigated whether the induction of HSP70 was dependent on the concentration of PGA1. Confluent monolayers of 37RC cells were infected with SV and treated with different concentrations of PGA1, starting 1 h p.i. After 24 h, an equal amount of protein from each sample was separated by SDS-PAGE and processed for immunoblot analysis; at the same time the supernatants were collected for virus titration. Results shown in Fig. 2(d) demonstrate that the synthesis of HSP70 was dependent on the concentration of PGA1 and substantial accumulation is found with doses that cause a dramatic block of virus replication (at 24 h, control, 32 HAU/2 × 10⁵ cells; treatment with 4 μg or 8 μg PGA1, negative).

To investigate the extent of PGA1-induced synthesis of HSP70 as compared to synthesis after heat shock, 37RC cells (2 × 10⁵ cells/ml/well) were either heat-shocked (42 °C for 20 min) or kept at 37 °C (control). After 10 min, medium was removed, fresh medium supplemented with 5% FCS at 37 °C was added and cells were kept at 37 °C for 24 h. Cells that had not undergone

Fig. 3. Effect of heat shock or PG treatment on HSP70 synthesis. 37RC cells were either heat-shocked (42 °C for 20 min; lane 1), or treated with 4 μg/ml PGA1 (lane 3), 4 μg/ml PGJ2 (lane 4) or control diluent at 37 °C (lane 2). After 24 h, an equal amount of protein from each sample was separated by SDS-PAGE and processed for immunoblot analysis using the anti-HSP MAb. Induction of HSP70 by PGs was comparable to, and in the case of PGJ2 greater than, that obtained after heat shock.
HSP70 induction by antiviral prostaglandins

Fig. 4. Kinetics of HSP70 synthesis after induction by PGA1. Confluent monolayers of 37RC cells were pulse-labelled for 1 h with [35S]methionine at various times after the addition of PGA1 (5 μg/ml) or control diluent. (a) [35S]Methionine incorporation into TCA-insoluble material from untreated (○) or PGA1-treated cells (●). Data represent the mean ± s.d. of duplicate samples. (b) SDS-PAGE analysis and autoradiography of samples treated as described in (a). Protein from each sample containing the same amount of radioactivity was loaded onto gels. Lanes 1 to 12, proteins pulse-labelled 0, 1, 2, 4, 6, 8, 12, 24, 28, 32, 36 and 48 h after PGA1 treatment. HSP70 synthesis (indicated by the arrow) was maximal between 8 and 12 h after treatment. (c) To determine the amount of HSP70 synthesis and degradation at various times after PGA1 treatment, confluent monolayers of 37RC cells were treated with PGA1 (5 μg/ml) or control diluent and collected at the beginning of treatment (lane 1) and after 3, 6, 12 or 24 h (lanes 2 to 5). An equal amount of protein from each sample was processed for immunoblot analysis after SDS-PAGE separation. (d) In a parallel experiment, HSP70 synthesis at different times after PGA1 treatment was quantified in uninfected or SV-infected, [35S]methionine-labelled cells, after immunoblot analysis, by cutting out the relevant bands from the blots and counting the radioactivity in a β-scintillation counter. ○, Uninfected; ●, PGA1-treated; △, SV-infected; ▲, SV-infected, PGA1-treated cells. (e) 37RC cells, pretreated with PGA1 for 24 h, were washed five times to remove PGA1 and collected immediately after washing (lane 1), or 3, 6, 12 and 24 h after PGA1 removal (lanes 2 to 5). An equal amount of protein from each sample was processed for immunoblot analysis after SDS-PAGE separation. HSP70 was found to be stable for at least 24 h after PGA1 removal.

Kinetics of synthesis and turnover of HSP70 after induction by PGA1

Heat shock were treated with 4 μg/ml PGA1, 4 μg/ml PGJ2 or control diluent. After 24 h cells were lysed, and an equal amount of protein from each sample was separated by SDS-PAGE and processed for immunoblot analysis. Fig. 3 shows that the induction of HSP70 by the PGs is comparable to, and in the case of PGJ2 greater than, that obtained after heat shock.

To examine the kinetics of HSP70 synthesis, confluent monolayers of 37RC cells were treated with PGA1 (5 μg/ml) or control diluent and, after a 15 min preincubation in methionine-free medium, pulse-labelled for 1 h
with [35S]methionine (2 μCi/2 × 10⁵ cells) at various times during the next 48 h. After being pulse-labelled, cells were collected, lysed in SDS sample buffer and, after determination of the radioactivity incorporated into TCA-insoluble material, radiolabelled proteins were separated by SDS–PAGE and processed for autoradiography. PGA₁ treatment caused a slight decrease in protein synthesis between 2 and 3 h after treatment; protein synthesis returned to control levels by 4 h and no significant alterations were noticed up to 48 h (Fig. 4a).

HSP70 synthesis started between 2 and 3 h after treatment, was maximal at 12 h and went back to control levels by 26 h after PGA₁ addition (Fig. 4b); no synthesis was detected 48 h after treatment (data not shown).

HSP70 synthesis after PGA₁ treatment was also studied by immunoblot analysis. Confluent monolayers of 37RC cells were treated with PGA₁ (5 μg/ml) or control diluent. Cells were collected at various times after the addition of PGA₁, lysed in lysis buffer and, after protein determination, an equal amount of protein from each sample was processed for immunoblot analysis after separation by SDS–PAGE. Fig. 4(c) shows that accumulation of p74 started 3 h after the addition of PGA₁ and continued for the following 24 h. In a parallel experiment, HSP70 synthesis was quantified in uninfected and SV-infected [35S]methionine-labelled cells at various times after PGA₁ treatment, after immunoblot analysis using the anti-HSP antibody, by cutting the relevant bands from blots and counting the radioactivity in a β-scintillation counter (Fig. 4d).

To investigate the stability of the PGA₁-induced HSP70, PGA₁ was removed from 37RC cells, pretreated for 24 h at a concentration of 5 μg/ml, by repeated washing and fresh culture medium was added. At different times after PGA₁ removal, samples were collected and, after protein determination, an equal amount of protein from each sample was processed for immunoblot analysis. HSP70 induced by PGA₁ was found to be extremely stable for at least 24 h after synthesis, as shown in Fig. 4(e).

**Discussion**

It has been shown that PGA antiviral activity in several virus–host cell models is associated with alteration of the synthesis and/or maturation of specific virus proteins. PGA treatment prevents the synthesis of three specific vaccinia virus (VV) polypeptides in mouse L fibroblasts (Santoro et al., 1982a). When cytoplasmic RNA from PGA-treated, VV-infected cells is translated in cell-free systems, similar selective inhibition of viral polypeptides is observed. The fact that PGA, even at much higher doses, does not exert any direct inhibitory action on transcription *in vitro* and has no effect on primary transcription and translation of VV RNA when assayed in coupled cell-free systems, suggests that the synthesis
and/or activation of a host product mediates the antiviral action (Benavente et al., 1984). In the same cell line, PGA treatment also specifically suppresses the synthesis of VSV glycoprotein G and alters its mobility in SDS–polyacrylamide gels, suggesting a possible action of PGA on virus protein glycosylation (Santoro et al., 1983).

When we studied the effect of PGA on the replication of SV in monkey kidney cells, we found that in the presence of PGA, three virus polypeptides, P, NP and M, were synthesized normally up to 48 h p.i., whereas the two viral glycoproteins, HN (M, 70K), with both haemagglutinin and neuraminidase activity, and the F protein (M, 64K), which plays an essential role in haemolysis, cell fusion and infectivity of the virion (Choppin & Scheid, 1980; Peluso et al., 1978), were present in an altered form of lower M, (66K and 63K, respectively). We have now demonstrated that these structural alterations, previously shown to be due to a defect in the glycosylation process which prevents the incorporation of HN into the host cell membrane (Santoro et al., 1989a), result in the block of virus maturation and budding from infected cells. PGA was first reported in 1982 (Santoro et al., 1982b).

Interest in the identification of this protein increased when, a few years later, we found that the synthesis of a polypeptide of the same M, was induced by PGA1 in a different cell line, human K562 erythroleukaemia (Santoro et al., 1986). Moreover, recent studies with actinomycin D revealed that as in VV, the PGA1-induced alteration of SV glycoproteins described above are mediated by a host product, and p74 appeared to be the best candidate for this role; we have now identified p74 as an HSP.

HSPs or stress proteins are a specific set of polypeptides, the synthesis of which is induced by heat shock or other environmental stresses; they can be divided into five families with M,s of 15K to 30K (low M, HSPs), 60K (HSP60), 70K (HSP70), 90K (HSP90) and 100K to 110K (HSP110) (Schlesinger et al., 1990). In the eukaryotic cell, HSPs are generally present as multigene families, consisting of closely related protein isoforms, with members being expressed in unstressed cells (constitutive HSPs) as well as following heat treatment (inducible HSPs) (Lindquist & Craig, 1988). The human HSP70 group has at least five distinct members, the genes of which have been mapped to chromosomes 6, 14 and 21 (Morimoto et al., 1990). The structure of HSPs has been highly conserved throughout evolution from bacteria to man, and HSP70 represents one of the most highly conserved proteins examined to date, with greater than 50% similarity (greater than 90% in some domains) between species as divergent as Escherichia coli and Homo sapiens (Hunt & Morimoto, 1985).

Although it is widely assumed that these proteins protect cells from the effect of stress, they have been implicated in embryogenesis, thermotolerance, protection of partially denatured proteins, regulation of cell growth and differentiation, and viral infection (for review see Schlesinger et al., 1990). Constitutive HSP70 proteins are essential for cell survival, are needed for the import of several proteins into eukaryotic cell organelles, and can bind to and, in the presence of ATP, dissociate protein complexes, including clathrin-coated vesicles, nucleolar proteins and a λ bacteriophage DNA replication complex (Schlesinger, 1990).

There is a growing body of literature which describes the relationship between HSPs and virus replication. Induction of HSPs during virus infection has been reported both in prokaryotic and eukaryotic cells. Bacteriophage infection of E. coli, in particular λ phage, activates HSP genes. DnaK protein, the product of the E. coli dnaK gene which is 50% identical to the drosophila HSP70, is found in a complex with phage proteins O and P, which are part of the λ phage DNA replication complex, whereas two other E. coli HSPs, the products of the groES and groEL genes, are involved in the assembly of the head proteins of bacteriophages λ and T4, and of the tail proteins of bacteriophage T5 (reviewed by Bond & Schlesinger, 1987).

Infection of monkey or mouse cells with simian virus 40 or polyoma virus has been reported to increase the synthesis of two host, heat-inducible 92K and 72K proteins (Khandjian & Turler, 1983), whereas infection of human cells with adenovirus increases the expression of hsp genes (Kao & Nevins, 1983). HSP induction by mutants of herpes simplex virus type 1 (HSV-1) has been demonstrated in chicken embryo fibroblasts (Notarianni & Preston, 1982) and the presence of abnormal forms of the HSV-1 immediate early polypeptide Vmw175 was found to be the signal for induction (Russell et al., 1987). Yura et al. (1987) have also shown that infection of human neuroblastoma cells with HSV-2 at 40 °C results in the expression of host cell stress proteins accompanied by a marked reduction of the synthesis of the late viral polypeptide ICP5 and of virus production, suggesting that the accumulation of HSPs may be involved in the arrest of virus growth and survival of the infected cell. Avirulent strains of Newcastle disease virus have been found to induce HSPs strongly in chicken embryo cells (Collins & Hightower, 1982), and the infection of these cells with SV stimulates the synthesis of several cellular polypeptides, including an 86K protein and two glucose-regulated proteins of M,s of 99K and 78K respectively (Peluso et al., 1978). The role of HSPs in virus replication, however, remains to be established. It may simply reflect a cellular stress response to the events following infection or it may interfere with virus replication.
We did not find any significant increase in HSP70 synthesis induced by SV in AGMK cells at different times p.i., but we did find that this protein was induced by PGA treatment in a situation where altered forms of virus proteins accumulate and virus replication is blocked. This situation mimics, by the use of an antiviral compound, that described for altered proteins of mutants of HSV-1 and HSV-2 (Russell et al., 1987; Yura et al., 1987).

Although we cannot exclude the possibility that HSP70 synthesis represents a cellular response to the exposure to PGs, the possibility is minimized by the fact that HSP70 is induced at concentrations which do not alter nucleic acid or protein synthesis in these cells, and which partially protect the cells from the virus-induced c.p.e.

The possibility that this protein could have a role in PGA antiviral activity is suggested by the following. (i) Only PGs that possess antiviral activity induce the synthesis of HSP70. (ii) Studies of HSP70 metabolism establish that the protein is present during virus protein synthesis. (iii) The accumulation of HSP70 above constitutive levels starts at concentrations of PGA which are effective against virus replication. (iv) Suppression of HSP70 synthesis by actinomycin D also abolishes the PGA-induced alterations in SV protein synthesis (Santoro et al., 1989).

The mechanism by which PGAs induce HSP70 synthesis is not known. Since the presence of abnormal or defectively glycosylated polypeptides is a well known signal for HSP induction (Ananthan et al., 1986; Pelham, 1986), it could be argued that HSP70 synthesis is induced in response to the accumulation of structurally altered forms of SV proteins after PGA treatment (Santoro et al., 1989). However, this hypothesis is contradicted by the fact that HSP synthesis after the addition of PGA precedes virus protein synthesis and is also induced in uninfected, uninfected cells. The fact that PGs of the A type have been shown to react in a covalent manner with sulfhydryl groups and to bind to cysteine-rich proteins (Ham et al., 1975) suggests that PGA could bind directly to specific cellular proteins, producing modifications of their tertiary structure, which could in turn induce HSP70 synthesis.

On the other hand a direct role for cyclopentenone PGs, which have been shown to be transported to the nuclei and to bind to nuclear proteins and DNA (Fukushima et al., 1989; Karmali et al., 1976), in the activation of heat shock gene expression cannot be excluded.

Finally we have shown that HSP synthesis is induced by cyclopentenone PGs in the human K562 erythro-leukaemic cell line (Santoro et al., 1989b) and in MT-2 human leukaemia cells (D’Onofrio et al., 1990b), and preliminary results have indicated that PGs of the A and J types can induce the synthesis of stress proteins in several other monkey, canine and human cell lines, in association with the inhibition of several DNA and RNA viruses, indicating that induction of HSP70 by PGA is a general response of mammalian cells to PGs.

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