Inhibition of Virus Protein Glycosylation as the Mechanism of the Antiviral Action of Prostaglandin A in Sendai Virus-infected Cells

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SUMMARY

Prostaglandin A (PGA) inhibits Sendai virus replication at doses non-toxic to uninfected cells. In this report, the antiviral action of PGA was found to be associated with specific alterations of viral protein synthesis. SDS-PAGE analysis of [35S]methionine-labelled proteins showed that while the non-glycosylated viral polypeptides (P, NP and M) were normally synthesized in PGA1-treated cells, the viral glycoproteins HN and F0 were not detected. Two new polypeptides of M, respectively 4000 and 1000 lower than the HN and F0 proteins were instead detected. The results suggest that these new polypeptides are defectively glycosylated forms of HN and F0. In fact PGA1 was found selectively to inhibit glucosamine incorporation into Sendai virus-infected cells, but not in uninfected cells. Moreover, in infected cells the inhibition of glucosamine incorporation appeared to be selective towards viral polypeptides. This effect was not due to a decreased uptake of glucosamine from the cells after PGA1 treatment. The results also show that the PGA1-induced alteration of the HN protein caused a loss of its biological function and prevented the insertion of this protein into the cell membrane, thereby blocking virus maturation. Finally, a polypeptide of M, 74K, the synthesis of which was induced by PGA1, appeared to be a possible mediator of PGA1 antiviral action.

INTRODUCTION

Since their discovery, prostaglandins (PGs), a class of naturally occurring cyclic 20-carbon fatty acids synthesized by most types of cells, have been shown to be involved in the regulation of many physiological phenomena, including immune function (Goodwin & Webb, 1980), interferon action (Pottathil et al., 1980) and the control of cell growth and differentiation (Santoro & Jaffe, 1989). Experimental evidence for a relationship between PGs and virus replication has been accumulating over the past few years (for review, see Santoro, 1987).

Transformation of BALB/c 3T3 mouse fibroblasts with simian virus 40 (Ritzi & Stylos, 1976) or polyoma virus (Hammarström, 1977), as well as infection of human synovial fibroblasts with several viruses, including rubella virus, measles virus, Newcastle disease virus and adenovirus (Yaron et al., 1981), have been shown to increase PG biosynthesis by these cells. On the other hand, treatment with exogenous PGs has been shown to influence virus replication (Santoro, 1987).

We have previously shown that PGs of the A series (PGAs) are potent inhibitors of virus replication in several systems, among which are Sendai virus (SV) in African green monkey kidney (AGMK) and HEp-2 cells (Santoro et al., 1980, 1983b), vaccinia virus (VV) and vesicular stomatitis virus (VSV) in mouse L fibroblasts (Santoro et al., 1982a, 1983a). In the case of SV, PGA also completely prevented the establishment of persistent infections (Santoro et al., 1980).
The antiviral action was found to be pharmacological and specific for PGAs (both PGA₁ and PGA₂), while PGs of the B, E and F series, prostacyclin, 6-keto PGF₁α, and thromboxane B₂ were inactive.

In all systems, the action of PGA was dose-dependent, able to decrease virus production by more than 90% at non-toxic doses, while in uninfected cells it did not cause significant changes in the uptake of precursors or in the synthesis of DNA, RNA and proteins (Santoro, 1987).

In mouse L fibroblasts, PGA treatment prevented the synthesis of three specific VV polypeptides (Santoro et al., 1982a, 1983a; Benavente et al., 1984), and strongly suppressed the synthesis of glycoprotein ‘G’ of VSV (Santoro et al., 1983a). Moreover, PGA altered the mobility of this protein in SDS–polyacrylamide gels, producing an apparent decrease in its Mr of about 4K. A possible action of PGA on virus protein glycosylation was hypothesized (Santoro et al., 1983a).

In the present report we describe the effect of PGA on treatment of cellular and viral protein synthesis and glycosylation, in an AGMK cell line (37RC), infected with SV. The results indicate that, in this system, the antiviral action of PGA is associated with alterations of specific viral proteins, and that this effect is probably mediated by a host polypeptide. More importantly, PGA₁ was found to be a specific inhibitor of SV protein glycosylation.

**METHODS**

**Cell cultures.** The establishment and culture of AGMK 37RC cells have been described in detail (Santoro et al., 1981). Briefly, the 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. This cell line produces interferon upon induction with Newcastle disease virus. Cell counts were performed using a haemocytometer and viability was determined by vital dye (0.02% trypan blue) exclusion.

**Prostaglandins.** PGA₁ (Sigma) was stored as a 100% ethanolic stock solution (10 mg/ml) at −20°C, and was diluted to the appropriate concentration at the time of use. Control media contained the same concentration of ethanol diluent (0.04%), which was shown not to affect cell metabolism. When determined by radioimmunoassay PGA₁ is stable in tissue culture medium for at least 24 h.

**Virus infection.** Stocks of SV (obtained from Dr R. Calio, Department of Experimental Medicine, II University of Rome, Italy) were prepared by allantoic inoculation of 10-day-old embryonated eggs with 0.2 ml of a 1:1000 dilution of infected allantoic fluid, which was harvested after 72 h at 37°C, clarified by centrifugation at 4000 g for 10 min and stored at −80°C. Confluent cell monolayers were washed with phosphate-buffered saline (PBS) and then virus samples were added (0.5 ml for Linbro wells and 2 ml for the flasks). After incubation for 1 h at 37°C, the 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 titrations.** Virus production was determined by measuring either the haemagglutinin units (HAU) present in the medium or the haemadsorption (HAD) by infected monolayers of 37RC cells. Haemagglutinin titrations were done according to standard procedures. For HAD measurements, cell monolayers were washed three times with PBS, and 0.5 ml of 0.1%, human type O Rh+ red blood cells was added. After incubation at 4°C for 60 min, cell monolayers were washed extensively with PBS and, after cell lysis, the number of erythrocytes adsorbed to AGMK cells was quantified by haemoglobin determination using a modification of the technique of Crosby & Furth (1956).

**Protein synthesis and glycosylation.** The kinetics of protein synthesis and glycosylation in uninfected and SV-infected confluent cells were studied in 24-well Linbro plates. At different times (see text) cells were labelled with 5 μCi/ml [³⁵S]methionine or 10 μCi/ml [6-[³²H]glucosamine hydrochloride (Amersham) and the radioactivity incorporated into acid-soluble and -insoluble material was determined as previously described (Santoro et al., 1982a). For protein synthesis measurements cells were kept for 15 min in methionine-deprived culture medium, before labelling. Briefly, cells were washed three times with PBS and 0.4 ml 5% TCA was added to each culture. After 1 h, 0.2 ml volumes of acid-soluble material were counted in Aquasol (New England Nuclear). Acid-insoluble radioactivity was measured after washing the TCA precipitates three times 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. Samples (0.2 ml) were counted in Aquasol.

**PAGE.** Confluent monolayers of cells were labelled with [³⁵S]methionine (10 μCi/ml) or [³²H]glucosamine (10 μCi/ml) at different times after infection with SV. Methionine-deprived medium was used for [³⁵S]methionine labelling and the appropriate length pulse-labellings were used for each experiment. After labelling, 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 as previously described (Santoro et al.,
PGA inhibits viral protein glycosylation

Among the different PGs and PG-related compounds tested (PGA₁, PGA₂, PGF₂α, PGE₂, PGF₁α, prostacyclin, thromboxane B₂, 6-keto PGF₁α, arachidonic acid, and two synthetic analogues, 16,16-dimethyl-PGE₂ methyl ester and 16,16-dimethyl-PG₁₂ methyl ester), only the PGs of the A series are potent inhibitors of SV production (Santoro et al., 1980). As anticipated (see Introduction), PGA antiviral action was dose-dependent, and the dose of 4 μg/ml, which was shown to be the most effective non-toxic dose, causing 94 to 100% inhibition of SV replication (Santoro et al., 1981), was used in all subsequent experiments. Confluent monolayers of 37RC cells were infected with egg-grown SV (10 HAU/10⁵ cells) 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. After 48 h SV caused a severe c.p.e. in 37RC cells. PGA₁ treatment generally delayed the appearance of the c.p.e. but only partially protected the host cells; however, it totally suppressed virus replication as measured by haemagglutination activity in the medium (control, 96 ± 0 HAU/2 × 10⁵ cells; PGA₁, negative, n = 3).

For protein synthesis measurement, cells were labelled with [³⁵S]methionine (10 μCi/well) for 2 h at different times before and after infection (Fig. 1). The radioactivity incorporated into acid-insoluble material was determined as described in the previous section. SV infection progressively inhibited the host protein synthesis [13% inhibition at 24 h and 35% inhibition at 48 h post-infection (p.i.)]. PGA₁ (4 μg/ml) was shown not to alter protein synthesis in uninfected cells, and to slightly, but not significantly, decrease protein synthesis in SV-infected cells, up to 48 h p.i.

The pattern of host and viral protein synthesis was analysed after labelling with [³⁵S]methionine, by separation in SDS–polyacrylamide gels and autoradiography. Confluent monolayers of cells were infected with SV as described above. PGA₁ treatment was started after the 1 h infection period and continued for the following 18 h. After this time cells were kept in methionine-deprived medium for 1 h, then labelled with [³⁵S]methionine (1 h pulse, 10 μCi/2 × 10⁵ cells), and processed as described in Methods. Even though PGA₁ did not alter the general pattern of the proteins synthesized, it did induce the synthesis of a new protein (PG protein) of approximate Mr, 74K (p74, Fig. 2). The synthesis of this protein has been previously described to start as soon as 2 h after PGA₁ administration (Santoro et al., 1982b).
Fig. 1. Effect of PGA₁ on protein synthesis of uninfected and SV-infected 37RC cells. Cells were pulse-labelled with [³⁵S]methionine (2 h, 10 µCi/10⁵ cells) at different times p.i. Protein synthesis was measured as incorporation into TCA-insoluble material. Data represent the mean ± S.E.M. of duplicate samples. (●, ○) Controls, (▲, △) SV-infected cells; open symbols indicate the addition of PGA₁.

Fig. 2. Effect of PGA₁ on the synthesis of cellular and viral polypeptides. SDS-PAGE analysis of [³⁵S]methionine-labelled polypeptides of uninfected (a) and SV-infected (b) 37RC cells treated with PGA₁ (4 µg/ml), actinomycin D (3 µg/ml) or control diluent. Lanes 1, control; lanes 2, PGA₁-treated; lanes 3, actinomycin D present; lanes 4, actinomycin D and PGA₁ present. Viral proteins were identified on the basis of their Mr, and in relation to the position of viral marker proteins from unlabelled egg-grown purified SV. The arrows indicate the position of the 74K cellular protein whose synthesis was induced by PGA₁. HN and Fo viral glycoproteins were not detected after PGA₁ treatment. Two new bands (66K, indicated by the asterisk, and 63K) were instead found. The effect of PGA₁ on viral glycoproteins was suppressed by treatment with actinomycin D. Mr values are indicated to the left of each gel.
PGA inhibits viral protein glycosylation

Treatment with actinomycin D at 3 µg/ml for 18 h was not lethal to 37RC cells, which have been described to be resistant to this drug (Benedetto et al., 1975), but caused almost total inhibition of RNA synthesis and strongly inhibited (>60%) protein synthesis, causing a substantial alteration of the electrophoretic pattern of the proteins synthesized (Fig. 2a). Actinomycin D treatment completely suppressed the synthesis of the M, 74K protein, suggesting that a new molecule of mRNA for this protein was synthesized in response to PGA1 treatment (Santoro et al., 1982b).

PGA1 induces specific alterations in SV proteins

In SV-infected cells (Fig. 2b), PGA1 treatment did not inhibit host protein synthesis and, as with the control cells, induced the synthesis of the 74K protein. PAGE of [35S]methionine-labelled proteins showed that PGA1 did not significantly inhibit the synthesis of the three virus polypeptides P, NP and M, while the two virus glycoproteins, HN (M, approx. 70K in our gel system) which possesses both haemagglutinating and neuraminidase activity, and F0 (M, 64K) the precursor of the active F1,2 protein with cell-fusing and haemolysing activities (Choppin et al., 1981), were apparently absent. However, two additional bands corresponding to proteins of M, 66K and 63K were found in PGA-treated infected cells. This was also demonstrated by densitometric analysis (data not shown).

In virus-infected cells actinomycin D treatment, started soon after infection and continuing for the next 18 h, almost totally suppressed cellular, but not viral RNA synthesis (SV RNA polymerase being resistant to actinomycin D; Santoro et al., 1981), as well as host protein synthesis, while SV proteins were synthesized normally (Fig. 2b). However, actinomycin D treatment strongly suppressed production of mature virions (unpublished data).

The effect of PGA1 on host and virus protein synthesis was completely suppressed by actinomycin D. In fact both the PGA1-induced production of the PG protein and the alterations in the synthesis of viral glycoproteins were abolished when the two were used simultaneously, suggesting that the synthesis of the 74K protein might be necessary for the effect of PGA1 on viral glycoproteins.

The effect of PGA1 treatment on SV protein synthesis was also studied by immunoblot analysis using a rabbit polyclonal anti-SV antibody. Confluent monolayers of cells were infected with SV and PGA1 treatment (4 µg/ml) was started after a 1 h period of infection. After 24 h cells were processed for SDS-PAGE and immunoblot analysis, as described in Methods. The same amount of protein was analysed for each sample. Fig. 3 shows the presence of four bands corresponding to the viral proteins P, HN, F0 and NP in SV-infected cells (lane 4). In PGA1-treated infected cells, the viral proteins P and NP were synthesized normally, whereas the bands corresponding to the HN and F0 glycoproteins were absent and two new viral proteins (M, 66K and 63K) were detected (Fig. 3, lane 5). The fact that these two new proteins were not present in PGA-treated non-infected cells suggests the possibility that these new bands could represent altered forms of the viral glycoproteins HN and F0. Moreover, the fact that the synthesis of the non-glycosylated viral polypeptides was not altered by this compound suggested that PGA1 could act by affecting protein glycosylation. Tunicamycin (TM), an inhibitor of protein glycosylation, has been shown to produce similar alterations in the synthesis of HN and F glycoproteins of SV in bovine kidney cells. Nakamura et al. (1982) have shown that treatment with TM (0.5 µg/ml), while not significantly affecting the synthesis of the non-glycosylated proteins P, NP and M, suppressed the synthesis of HN and F, and two new bands of M, 63K and 55K were detected, and determined to be the non-glycosylated forms of the HN and F proteins. In order to investigate whether PGA1 could have a TM-like effect on SV glycoproteins, uninfected and SV-infected cells were treated with TM (2 µg/ml), starting after the 1 h infection period, and cellular and viral proteins were analysed 24 h p.i. by immunoblot analysis (following SDS-PAGE) using a rabbit polyclonal anti-SV serum. Fig. 3 shows that, as reported for Madin–Darby bovine kidney cells, TM did not significantly affect the synthesis of P and NP SV proteins in AGMK cells. Synthesis of HN and F0 was not detected, but two new bands of M, 64K and 63K were revealed. While the 63K protein comigrated with the protein found after
PGA₁ treatment, the second polypeptide (which should have corresponded to the non-glycosylated form of HN) had an $M_r$ slightly lower (about 2K) than that induced by PGA₁.

Specific inhibition of glucosamine incorporation in SV-infected cells by PGA₁; effect on viral glycoproteins

The PGA-induced alterations in the synthesis of HN and F₀ described above, and the fact that the synthesis of the viral non-glycosylated polypeptides was not altered by this compound, suggested that PGA₁ could act by affecting protein glycosylation. The effect of PGA₁ treatment on the uptake and incorporation of [$^{3}$H]glucosamine in uninfected and SV-infected 37RC cells was then studied. Confluent monolayers of cells were infected with SV (10 HAU/10⁵ cells) for 1 h at 37 °C. After this time PGA₁ treatment (4 µg/ml) was started (time 0), and cells were labelled with [$^{3}$H]glucosamine (5 µCi/ml/10⁵ cells, 3 h pulses). Uninfected cells were treated and labelled identically. The uptake of [$^{3}$H]glucosamine by the cells and the incorporation into acid-insoluble material was determined at different times after PGA₁ treatment, as described in
PGA inhibits viral protein glycosylation

Methods. Fig. 4 shows that, in the first 6 h p.i. there was no difference in \[^{3}H\]glucosamine uptake and incorporation between uninfected and SV-infected cells, either untreated or treated with PGA1. However, after 24 h of infection with SV there was a lower level of \[^{3}H\]glucosamine incorporation (32\%) compared to the control. At the dose used PGA1 did not significantly affect \[^{3}H\]glucosamine incorporation in uninfected cells, but strongly inhibited it in cells infected with SV (48\% inhibition as compared to infected untreated cells; Fig. 4b). Moreover, this inhibition was not due to a decreased uptake of \[^{3}H\]glucosamine by 37RC cells, since at this dose PGA1 did not significantly alter \[^{3}H\]glucosamine uptake in uninfected cells, yet slightly increased it in infected cells, 24 hours p.i. (Fig. 4a).

In order to establish whether PGA1 was able to alter viral protein glycosylation, \[^{3}H\]glucosamine-labelled proteins were analysed by SDS–PAGE. Confluent monolayers of 37RC cells grown in 35 mm Petri dishes were infected with SV, as described in the previous experiment. PGA1 treatment (4 \(\mu\)g/ml) was started 1 h after virus infection and continued for the next 24 h. \[^{3}H\]Glucosamine labelling (10 \(\mu\)Ci/ml/2 \(\times\) \(10^5\) cells) was started at the same time and, after 24 h, samples were processed for SDS–PAGE and autoradiography as described in Methods.

Fig. 5(a) shows the electrophoretic pattern of \[^{3}H\]glucosamine-labelled proteins and the appearance of the two viral glycoproteins HN and F0 in SV-infected cells. SV infection appeared to alter 37RC glycoprotein synthesis (M. G. Santoro et al., unpublished data). When equal amounts of proteins in each sample were applied to the gels the bands corresponding to HN and F0 viral glycoproteins were not detectable in PGA1-treated samples (data not shown). When equal amounts of radioactivity were applied, glucosamine incorporation into host cell proteins was found to have been increased by PGA1, but was dramatically reduced in the two viral glycoproteins (Fig. 5a, b). After taking into consideration the amounts of protein applied to the gels, these data suggest that about 10 to 20\% of glycosylated HN and F0 is still synthesized after PGA1 treatment. The fact that \[^{3}H\]glucosamine-labelled viral glycoproteins of lower Mr were not detected, indicates that a block in an early step of glycosylation is probably occurring. Similar results were observed in the glycosylation of the ‘G’ protein of VSV after interferon treatment in mouse L cells (Maheshwari et al., 1980). Finally, high Mr, host cell glycoproteins were not affected by PGA1 treatment, even in virus-infected cells.
Fig. 5. Effect of PGA\textsubscript{1} treatment on glycoprotein synthesis. (a) SDS-PAGE analysis of \textsuperscript{[3}H\textsuperscript{]}glucosamine-labelled glycoproteins from untreated (lanes 1 and 3) or PGA\textsubscript{1}-treated (lanes 2 and 4) cells, uninfected (lanes 1 and 2) or 24 h after SV infection (lanes 3 and 4). PGA\textsubscript{1} specifically inhibited the synthesis of viral glycoproteins HN and F\textsubscript{0}. (b) Densitometric analysis of control (traces 1 and 3) and PGA\textsubscript{1} (traces 2 and 4) autoradiographic patterns shown in (a). Direction of migration is indicated by the arrow at the bottom of the figure. Bars at the bottom of the figure indicate the position of viral markers F, HN, NP, F and M from unlabelled egg-grown purified SV. Mr values are indicated to the left in (a).

**PGA\textsubscript{1}-induced structural alteration of the HN protein inhibited its incorporation into the cell membrane**

To investigate further whether the structural alteration and the defect in the glycosylation process could impair HN maturation and its insertion into the cell membrane, two different approaches were undertaken. HAD tests were performed to assess the presence of biologically active HN on the membrane of 37RC cells infected with SV. HAD units were measured 24 and 48 h p.i. in SV-infected cells (10 HAU/10\textsuperscript{5} cells) and treated with PGA\textsubscript{1} (4 \mu g/ml) as described above. The results demonstrate that PGA\textsubscript{1} treatment was able to decrease HAD by more than 75% in this system [control 0.47 ± 0.01, PGA\textsubscript{1} 1.08 ± 0.02, infected control (24 h) 29.5 ± 2.5, infected PGA\textsubscript{1}-treated (24 h) 6.88 ± 0.8 \mu g haemoglobin/2 × 10\textsuperscript{5} cells]. The lack of HAD activity in SV-infected cells after PGA\textsubscript{1} treatment, could be because the structural alteration produced by PGA\textsubscript{1} resulted either in an inactive HN protein or in a block of its incorporation into the cell membrane.

In order to investigate whether PGA\textsubscript{1} treatment could inhibit incorporation of the HN protein into the cell membrane, surface glycoproteins of confluent monolayers of uninfected or SV-infected 37RC cells were labelled with NaB\textsubscript{3}H\textsubscript{4} after treatment with neuraminidase and galactose oxidase, as described in Methods. Confluent monolayers of 37RC cells were infected with SV and treated with PGA\textsubscript{1}, as previously described. Surface glycoproteins were labelled 18 h p.i. Electrophoretic patterns of \textsuperscript{3}H-labelled proteins are shown in Fig. 6. The appearance of a new labelled protein, whose Mr (approx. 70K) corresponded to the HN viral glycoprotein, was evident in SV-infected cells. The appearance of this protein in infected cells is almost completely prevented by PGA\textsubscript{1} treatment, suggesting that the PGA\textsubscript{1}-induced alterations of the HN protein
PGA inhibits viral protein glycosylation

Fig. 6. PGA₁ treatment inhibits HN glycoprotein expression on the AGMK cell plasma membrane. SDS-PAGE analysis of cell surface-associated glycoproteins labelled with NaB₃H₄ after neuraminidase and galactose oxidase treatment. Uninfected cells, control (lane 1) and treated with PGA₁ (lane 2); SV-infected cells, control (lane 3) and treated with PGA₁ (lane 4). A new band, whose Mr corresponded to the HN glycoprotein, is shown in SV-infected cells, 24 h p.i. (lane 3). Treatment with PGA₁ (4 µg/ml) prevented the appearance of this band (lane 4). Mr values were calculated in relation to the position of viral marker proteins from unlabelled, egg-grown, purified SV as in Fig. 5. Mr values are indicated to the left.

As has been described for many other aspects of PG action, different types of PGs produce different effects on virus replication in several virus–host systems, in relation to the dose and the structure of the cyclopentane ring of the molecule.

Depending on the dose, PGE₂ and PGF₂α were shown to increase the size of herpes simplex virus (HSV) plaques in Vero cells (Harbour et al., 1978; Baker et al., 1982) but not in human fibroblasts (Trofatter & Daniels, 1980). Several PGs (PGA₁, PGB₁, PGE₁ and PGF₂α) have been reported to enhance basal as well as dexamethasone-stimulated replication of murine mammary tumour virus in mouse mammary adenocarcinoma (Svec et al., 1982; Karmali et al., 1982). On the other hand, PGs of the E series were shown to inhibit the production of
parainfluenza virus type 3 in WISH cells (Luczak et al., 1975), measles virus in Vero cells (Dore-Duffy, 1982), and Mengo virus, MM virus and poliovirus in L929 fibroblasts and HeLa cells (Giron, 1982).

For several years we have studied the role of PGs in the control of virus replication and, as mentioned in the Introduction, have found that PGAs are potent inhibitors of virus replication in several models, including SV, VSV, VV, HSV types 1 and 2 (Santoro, 1987) and human T cell leukaemia/lymphoma virus type I (D'Onofrio et al., 1987). Recently we have also shown that a long-acting synthetic analogue of PGA2 (16,16-dimethyl-PGA2 methyl ester) can suppress influenza A virus replication in mice (Santoro et al., 1988). PGs of series A, but not B, D, E or F, were also shown to potently suppress encephalomyocarditis virus replication in mouse L cells (Ankels et al., 1985).

SV, a negative-stranded RNA virus, grown in AGMK cells, was the first model in which PGA antiviral activity was discovered (Santoro et al., 1980). In this system, both PGA1 and PGA2 were able to inhibit virus replication up to 100% at doses that were non-toxic to the cells and did not affect DNA, RNA or protein synthesis in uninfected cells. The antiviral action was specific for PGAs and was found not to be mediated by cAMP. Pretreatment of either the host cell or the virus before infection had no effect; the fact that treatment during the infection period or for a short time post-infection (up to 3 h) did not cause any change in virus replication indicated that PGA did not act at an early stage (i.e. adsorption, entry or uncoating) in the process of virus replication (Santoro et al., 1980). Moreover, no effect on the synthesis of virus (actinomycin D-resistant) RNA was observed after PGA treatment (Santoro et al., 1981). As earlier studies on different virus models had indicated that the antiviral action of PGA is associated with alterations in the synthesis of specific virus proteins, in this paper we analysed the effect of PGA1 on SV protein synthesis and glycosylation.

SV, like other paramyxoviruses, consists of an inner nucleocapsid surrounded by a membranous envelope covered with spikes formed by two viral glycoproteins, the HN with both haemagglutinating and neuraminidase activity, and the F protein which plays an essential role in haemolysis, cell fusion and infectivity of the virion (Choppin & Scheid, 1980; Peluso et al., 1978).

The results described in this paper have shown that, in the case of SV, the antiviral action of PGA is also associated with specific alterations of protein synthesis and indicated that the only proteins whose synthesis was altered were the two viral glycoproteins HN and F0, whereas the non-glycosylated viral proteins and the host cell proteins were not affected. SDS–PAGE analysis in fact revealed that the viral HN and F0 glycoproteins were not detected in PGA1-treated infected cells, while two new viral proteins of Mr 66K and 63K, were found instead. These new polypeptides could represent either non-glycosylated forms of HN and F0, or products of proteolytic degradation of these proteins as a consequence of defective glycosylation.

These results are very similar to the effect of PGA1 on VSV glycoprotein ‘G’, which was shown to undergo a decrease in Mr of about 4K after PGA1 treatment. In the case of SV, we have shown a defect in the glycosylation process of viral proteins, as demonstrated by the dramatic inhibition of [3H]glucosamine incorporation only in SV-infected cells and specifically in the two virus glycoproteins HN and F0. This effect was not due to a decrease in the uptake of sugar by the cells. Even though the data described suggest an action of PGA1 on an early step of viral protein glycosylation (since no intermediate forms of glucosamine-labelled virus proteins were detected), this was shown not to be a TM-like effect.

It is interesting to find that the inhibition of glucosamine incorporation appeared to be specific for viral proteins, while host cell glycoproteins did not appear to be affected and there was no inhibition of glucosamine incorporation into uninfected cells.

Because both the HN and F proteins have been shown to contain fucose, mannose, galactose and glucosamine but no galactosamine or sialic acid, it has been suggested that all sugar chains in these glycoproteins should occur exclusively as asparagine-linked sugar chains (Yoshima et al., 1981). However, the oligosaccharide patterns of HN and F were found to be totally distinct, indicating that the sugar chains of the viral envelope glycoproteins were not simply a replica of
PGA inhibits viral protein glycosylation

host membrane glycoproteins; moreover, the complex-type sugar chains of both HN and F contain, in their outer chain moieties, a trisaccharide residue [Galβ1→4(Fucα1→3)GlcNAc], that is rarely found in animal glycoproteins (Yoshima et al., 1981). This could partially explain how PGA1 could selectively inhibit viral protein glycosylation without significantly affecting host cell glycoproteins.

A similar inhibition of [3H]glucosamine incorporation into the ‘G’ glycoprotein of VSV in mouse L cells has been reported to occur after interferon treatment (Maheshwari et al., 1980). We have previously described analogies in the mechanism of action of PGA and interferon in a different system, mouse L fibroblasts infected with VV (Santoro et al., 1983c).

Finally the results indicate that the PGA1-induced alteration in the structure of the HN protein inhibits both its function (HAD activity) and its incorporation into the host cell membrane, and could be responsible for the block of virus maturation in these cells.

The ability of PGA1 to interfere with the glycosylation of viral proteins is extremely interesting. However, the mechanism by which PGA1 can specifically prevent glycosylation of viral proteins, and whether this could be a general mechanism of PGA antiviral action, remains to be established. The possibility that the p74 protein, which is synthesized in these cells after induction by PGA1, could be involved in the antiviral action and, more specifically, could interfere with viral protein glycosylation is presently under study.

We have shown recently that PGJz, a dehydration product of PGD2 in aqueous solution (9-deoxy-A9-PGD2), also has a potent antiviral activity against SV in AGMK cells, being able to inhibit virus replication by up to 99% at doses not toxic to uninfected cells (Santoro et al., 1987). PGJz appeared to act in a very similar way to PGA1 in this system, both in the active dose range and in the mechanism of action, since it also induced the synthesis of p74 protein and produced similar alterations in the viral proteins synthesized. As both PGAs and PGJz possess an α,β-unaturated carbonyl group in the cyclopentanone ring of the molecule, we hypothesize that this molecular structure could be the feature necessary for the antiviral activity to occur.

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