Prostaglandin A Inhibits the Replication of Vesicular Stomatitis Virus: Effect on Virus Glycoprotein

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SUMMARY

Prostaglandins of the A series were found to strongly suppress the replication of vesicular stomatitis virus (VSV) in mouse L fibroblasts. The highest non-toxic dose of PGA1, 4 μg/ml, decreased VSV production by 93.6%. At this dose, PGA1 did not alter DNA, RNA or protein synthesis in uninfected L cells for periods up to 24 h, whereas it further suppressed protein synthesis and slightly increased RNA synthesis in VSV-infected cells. The presence of PGA1 during virus adsorption, with no treatment after infection, reduced VSV yields by 63.6%. However, the presence of PGA1 during an early step of VSV replication was not essential for the antiviral action to occur (PGA1 treatment could be started 1 to 2 h post-infection). Apart from a slight overall inhibition of virus protein synthesis, PGA1 strongly suppressed the synthesis of the VSV glycoprotein G; moreover, it produced an alteration in the mobility of this protein in SDS-polyacrylamide gels. We propose that this slight decrease in molecular weight (about 4000) of the G protein in the presence of PGA1 could be due to an alteration in the glycosylation process.

Prostaglandins (PGs) are a group of cyclic 20-carbon fatty acids that are synthesized by most types of cells and are involved in the control of many physiological phenomena, including cell growth and differentiation (Jaffe & Santoro, 1977), immune function (Goodwin & Webb, 1980) and interferon action (Pottathil et al., 1980). Recently PGs have also been shown to be involved in the control of virus replication. As for other aspects of prostaglandin action, different types of PGs have been reported to produce different effects on viral replication in several virus-host systems. Prostaglandins of the E series (PGE) were shown to inhibit the production of parainfluenza 3 virus in WISH cells (Luczak et al., 1975). PGEs (in contrast to PGA and thromboxane B2) were also reported to decrease virus yields in measles virus-infected Vero cells (Dore-Duffy, 1982). On the other hand, PGE and PGF increased the size of plaques and, at low multiplicity of infection (m.o.i.), the yield of herpes simplex virus (HSV) in Vero cells (Harbour et al., 1978). Finally, no effect on virus replication was reported in human cells infected with HSV after treatment with prostaglandins of the E, A and F series (Trofatter & Daniels, 1980).

In previous studies, we have demonstrated that prostaglandins of the A series are potent inhibitors of Sendai virus and vaccinia virus replication, respectively, in African green monkey kidney cells (Santoro et al., 1980, 1981) and mouse L fibroblasts (Santoro et al., 1982). In both cases, the antiviral activity was specific for prostaglandins of the A series, and dose-dependent. In the case of Sendai virus, PGA prevented the establishment of persistent infection.

In the present report, we describe the effect of PGAs on the replication of vesicular stomatitis virus (VSV) in mouse L cells. PGAs were found to strongly suppress virus replication in this system, as in the two others. Possible mechanisms of this antiviral action were investigated.

Mouse L fibroblasts were grown in monolayers, as previously described (Santoro et al., 1982). These cells normally synthesize PGE, as measured by radioimmunoassay after organic solvent extraction and silicic acid chromatography (Jaffe et al., 1973). VSV, strain Orsay, was grown in

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Table 1. Inhibition of VSV replication in L cells by PGA₁ treatment

<table>
<thead>
<tr>
<th>VSV, p.f.u. x 10⁷/ml</th>
<th>Control</th>
<th>PGA₁</th>
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<tr>
<td>Protocol A</td>
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<tr>
<td>4.72 ± 0.24 (n = 8)</td>
<td>0.30 ± 0.01† (n = 8)</td>
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<tr>
<td>Protocol B</td>
<td></td>
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<tr>
<td>3.64 ± 0.20 (n = 4)</td>
<td>1.24 ± 0.01† (n = 4)</td>
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* Protocol A: PGA₁ treatment (4 µg/ml) was started after the 1 h adsorption period and continued for 24 h post-infection. Protocol B: cells were treated with PGA₁ only during virus adsorption. Virus yields were collected 24 h post-infection, diluted to 10⁻⁴, 10⁻⁵ and 10⁻⁶, and titrated by plaque assay on Vero cells. Four dishes were tested for each group at each dilution. Dishes were stained with crystal violet after 36 h and plaques were counted. † P < 0.01.

mouse L cells and purified by banding in sucrose gradients as previously described (Baxt & Bablanian, 1976). Confluent monolayers of L cells were infected at time 0 with 10 p.f.u. of VSV per cell, for 1 h at 37 °C, after which time the virus inocula were removed and media containing PGA₁ or ethanol diluent were added. Both media contained the same amount of ethanol diluent (0.04%); this had been shown not to affect either cell or virus metabolism. As determined by radioimmunoassay, PGA₁ is stable in tissue culture medium for at least 24 h.

Under the conditions used, infection of L cells with VSV caused a rapid 'shut-off' of host RNA and protein synthesis (see below), while it did not significantly alter PGE synthesis by L cells either at early (2 h post-infection, control = 1.82 ± 0.28; VSV-infected = 2.07 ± 0.09 ng/ml) or at late (24 h, control = 3.24 ± 0.84; VSV-infected = 3.98 ± 0.83 ng/ml) times after infection. Treatment of VSV-infected cells with PGA₁ (4 µg/ml), starting after the 1 h adsorption period, caused a 1 to 3 h delay in the appearance of the cytopathic effect normally evident by 3 to 4 h under these conditions. After 24 h post-infection, there were no differences either in the cytopathic effects or in the rate of cell death in control and PGA₁-treated cells. At this time, supernatants from infected cells were collected for virus titration on Vero cells as previously described (Santoro et al., 1982).

Treatment with 4 µg/ml PGA₁, the most effective non-toxic dose, caused 93.6% inhibition of virus production (Table 1, A), and was thus used in all subsequent experiments. No difference in plaque size was noted. Since in this series of experiments, PGA₁ treatment was started after the 1 h adsorption period and it was effective when it was also started as late as 2 h after infection, the antiviral activity of PGA₁ did not seem to be related to an early event in virus replication. Nevertheless, the effect of PGA₁ treatment on virus adsorption was studied. For this purpose, L cells were grown to confluence in 60 mm Petri dishes and were infected with 0.5 ml of VSV suspension (calculated to give an average of 150 to 300 plaques) containing either PGA₁ (4 µg/0.5 ml) or control diluent. After 1 h of adsorption at 37 °C, virus inocula were removed and the monolayers were washed three times with minimal essential medium (MEM) to remove PGA₁. Dulbecco's modified MEM containing 3% newborn calf serum and 0.9% agar (5 ml/plate) was added and cells were incubated for 24 h. The presence of PGA₁ during virus adsorption was found to decrease the number of virus plaques by 63.6% (Table 1, B).

Treatment of confluent cell monolayers with PGA₁ (4 µg/ml/10⁶ cells) for periods up to 24 h was not toxic to uninfected L cells and did not alter either the uptake of precursors or the synthesis of DNA, RNA or proteins in these cells (as measured by the incorporation of [³H]thymidine, [³H]uridine and [³⁵S]methionine, respectively; Santoro et al., 1982). Fig. 1 shows the effect of the same dose of PGA₁ on the uptake of precursors (Fig. 1a and c) and the synthesis (Fig. 1b and d) of RNA and protein in VSV-uninfected L cells at different times. PGA₁ treatment produced a slight increase in [³⁵S]methionine uptake up to 3 h after infection, and further potentiated the virus-mediated 'shut-off' of protein synthesis, decreasing methionine incorporation into proteins at 4 and 5 h. No significant difference in the uptake of [³H]uridine was noted, whereas PGA₁ partially enhanced the rate of RNA synthesis at 3 h.
Fig. 1. Effect of PGA₁ on the uptake of precursors and synthesis of RNA and protein by VSV-infected L cells. Monolayers of L cells were infected with 10 p.f.u./cell of VSV and treated with PGA₁ (4 μg/ml/10⁶ cells) or control diluent. Cells were labelled (10 min. pulses) with 10 μCi/ml of [³⁵S]methionine (a and b) or [³H]uridine (c and d), as previously described (Santoro et al., 1982). Control uninfected cells treated with either PGA₁ or diluent were similarly labelled. Results are expressed as percent of uptake (a and c) or incorporation (b and d) in uninfected cells. For each point, data represent the mean of at least duplicate samples. ●, Control; ○, PGA₁.

We have previously demonstrated that PGA₁-mediated inhibition of vaccinia virus replication in the same cell line was related to the inhibition of some viral polypeptides. In order to investigate whether PGA₁ acted via a similar mechanism in VSV-infected cells, we analysed host and virus protein synthesized by separation of proteins on SDS-polyacrylamide gels (SDS-PAGE) and autoradiography after labelling with [³⁵S]methionine. PGA₁ did not alter the pattern of protein synthesis in uninfected L cells (Santoro et al., 1982). Fig. 2 shows the effect of PGA₁ (4 μg/ml) treatment on VSV-infected L cells at 5 (Fig. 2a) and 6 (Fig. 2c) h after infection. As previously shown in Fig. 1, PGA₁ produced a slight general inhibition of virus protein synthesis; however, the synthesis of the glycoprotein (G) was by far the most potently inhibited of all the virus proteins. More interestingly, slightly altered mobility (equivalent to a mol. wt. difference of approx. 4000) of the VSV G protein was observed in [³⁵S]methionine-labelled cell extracts from PGA₁-treated cells; this was also demonstrated by the densitometric analysis shown in Fig. 2(b). An alteration in the production of two low mol. wt. polypeptides was also noted.

The present study has shown that alterations of the host cell membrane caused by VSV entry and budding from L cells did not significantly activate cellular prostaglandin biosynthesis. Vaccinia virus has also been similarly shown not to influence PG synthesis in these cells unless cells were pretreated with IFN (unpublished results). Furthermore, we demonstrated that PGAs, which we have previously shown to inhibit the replication of Sendai and vaccinia viruses, also potently inhibit VSV production in L cells, indicating that the antiviral effect of
PGA has a broad spectrum of action. At the maximal non-toxic effective dose, PGA did not alter cell metabolism in uninfected cells, while it further suppressed protein synthesis and slightly increased RNA synthesis in VSV-infected L cells.

The antiviral action of PGA seems to be effective at two separate levels. The presence of PGA during virus adsorption, with no treatment after infection, reduced VSV production by 63.6%. However, the antiviral effect (93.6% inhibition) was also achieved when PGA treatment was started as late as 1 to 2 h post-infection, but continued through the length of the experiment; this indicates that an effect on an early step of virus replication was not necessary for the antiviral action of PGA. Apart from a slight overall inhibition of VSV protein synthesis, PGA treatment strongly suppressed the synthesis and slightly altered the mobility (during SDS-PAGE) of the virus G glycoprotein by an amount equivalent to approx. 4000 mol. wt. The slight decrease in mol. wt. of the G protein in PGA-treated cells might be due to an alteration of the glycosylation process. In fact, similar alterations in the mobility of the VSV G protein have been reported in the presence of an inhibitor of glycosylation, tunicamycin (TM), in the same cells (Maheshwari et al., 1980) and in Chinese hamster ovary (CHO) cells (Chatis & Morrison, 1983).
Schmidt & Schlesinger (1979) have shown that the G protein of VSV binds 1 to 2 moles of fatty acid (predominantly the methyl ester of palmitic acid) per mole of protein. This binding is a post-translational event and the fatty acids attach to the G polypeptide chain during the process of maturation, probably during the transfer of the glycoprotein to the cell membrane. Glycosylation of the G polypeptide does not necessarily precede the attachment of the fatty acid moiety. Because of the chemical nature of prostaglandins, we hypothesize that PGA₁ could bind to the G polypeptide, thus competing with the natural fatty acid precursor, altering the G protein structure and inhibiting glycosylation. The possibility that PGA₁ might bind directly to the virus glycoprotein is also suggested by the PGA₁-mediated inhibition of virus adsorption to the cell membrane. This possibility is currently under study.

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REFERENCES


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