Antiviral Activity of Prostaglandin A on Encephalomyocarditis Virus-infected Cells: A Unique Effect Unrelated to Interferon

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SUMMARY

Antiviral effects of prostaglandins of the A series (PGAs) on Sendai, vaccinia and vesicular stomatitis viruses have previously been reported and a relationship between the antiviral actions of PGAs and interferons has been suggested. We have investigated the antiviral activity of PGAs on encephalomyocarditis (EMC) virus. Using single-cycle assays of virus replication our results indicate that PGAs only inhibit when present in the culture medium after the cells are infected, and that they are most effective during incubation periods including from 3 to 5 h post-infection. Furthermore, viral RNA synthesis is blocked in infected cells treated with PGA and, as a result, viral antigens are greatly reduced in the cytoplasm of the cells 5 h post-infection. Since the antiviral effect of PGAs is unperturbed by actinomycin D, when cellular RNA synthesis is greatly reduced, it appears unlikely that induction of new cellular proteins is the reason for the antiviral activity of PGAs. In separate experiments we were unable to demonstrate directly the induction of interferon, or of the two dsRNA-dependent enzymes, 2',5'-oligoadenylate synthetase and protein kinase, which are greatly increased in interferon-treated cells. Thus, we conclude that the antiviral activity of PGAs is unrelated to the antiviral action of interferons and involves a unique mechanism independent of cellular protein synthesis.

INTRODUCTION

Prostaglandins of the A series (PGA₁, PGA₂) have been shown to inhibit replication of RNA and DNA viruses such as Sendai, vesicular stomatitis and vaccinia viruses (Santoro et al., 1980, 1981, 1982a, 1983a). The following observations have suggested a link between prostaglandin and interferon action. Increased levels of prostaglandins occur in the medium of interferon-treated cells (Yaron et al., 1977; Fitzpatrick & Stringfellow, 1980; Fuse et al., 1982). Inhibitors of cyclooxygenase known to block prostaglandin synthesis have been found to be inhibitory to the antiviral effects of interferon (Pottathil et al., 1980). A mouse cell line that is resistant to the biological effects of α and β interferons (L-1210R) also lacks fatty acid cyclooxygenase and is unable to synthesize prostaglandins in contrast to the interferon-sensitive parent cells (L-1210S) from which it originated (Chandrabose et al., 1981).

The antiviral effects of PGAs and interferons differ, however, in a fundamental way. Interferon action is greatly augmented and usually only detectable after preincubation of cultured cells with this antiviral agent prior to infection with virus. PGAs, on the other hand, have to be present in the medium during virus maturation to be effective and are ineffective when cells are only preincubated with these substances (Santoro et al., 1980). Therefore, an action of PGAs through induction of interferon seems unlikely.

In this report we have directly examined whether induction of interferon or other potential antiviral proteins that are implicated in interferon action is essential for the PGA effect. We used encephalomyocarditis (EMC) virus, an RNA virus whose inhibition in interferon-treated

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cells is believed to be caused primarily by activation of the 2',5'-oligoadenylate system (Baglioni et al., 1979). Since this virus replicates when cellular RNA synthesis is blocked by actinomycin D, it is feasible to determine whether cellular transcription plays a role in the antiviral effect of PGAs.

**METHODS**

**Materials.** All chemicals were from Sigma, except the following. Mouse α/β interferon was a gift of the late Kurt Paucker and had a specific activity of \(7 \times 10^7\) NIH Reference Units/mg (IU/mg). Radioactive 5-[\(\text{H}\)]uridine (30 Ci/mmol), [\(\text{S}\)]methionine (≥ 600 Ci/mmol), 32P-end-labelled 2',5'-oligoadenylate, and adenosine 5'\(\text{p}-32\text{P}\)-triphosphate (≥ 5000 Ci/mmol) were from Amersham. EMC virus was kindly provided by F. Besançon. Fresh human red blood cells, blood group O, were donated by one of the authors. Sera and culture media were from Gibco. Anti-mouse α/β interferon antibody (rabbit, 5 \(\times 10^5\) neutralizing units/ml) was purchased from Stratech Scientific Ltd., U.K. Anti-EMC virus serum was raised in rabbits against purified virus as described by Rueckert & Pallansch (1981). Goat anti-rabbit antibody coupled to fluorescein isothiocyanate was supplied by Medac, F.R.G.

**Viral assays.** Mouse L929 cells were routinely cultured in monolayers in Eagle's MEM supplemented with 10% foetal bovine serum, at 37°C in a 5% CO\(_2\) incubator. In single-cycle viral assays, confluent monolayers in 96-well microculture plates were infected with EMC virus at an m.o.i. of 50. After 1 h of infection at 37°C unadsorbed virus was decanted and the monolayers were rinsed once with MEM to remove residual serum and virus. Cells were subsequently exposed to individual prostaglandins in MEM at indicated concentrations for 6 h in routine assays, or, where time was varied, as indicated in the text. Individual prostaglandins were stored at \(-70°C\) in absolute ethanol at concentrations of 1 or 5 mg/ml. Solutions to be tested were prepared from such stocks immediately before use. Control experiments contained corresponding amounts of ethanol which were, however, without effect on L929 cells or virus yield. Virus determinations were done by haemagglutination of human erythrocytes of blood group O, using the procedure described by Jameson et al. (1977). Interferon assays were performed on L929 cells in 96-well plates, which were incubated for 24 h with serial twofold dilutions of interferon, using MEM plus 2% foetal bovine serum or 50 μg/ml bovine serum albumin as diluent. Cells were then washed once with MEM (or twice if residual prostaglandins had to be removed) and infected with 0.1 ml MEM-suspended EMC virus at an m.o.i. of 0.1. Interferon titres were determined 17 h later by virus yield reduction (Jameson et al., 1977).

**Antibody purification.** Commercial anti-mouse α/β-interferon antibody was purified using a 1 ml syringe containing 0.1 g Protein A–agarose beads preswollen in phosphate-buffered saline (PBS) (0.4 ml of gel, capacity approx. 10 mg human IgG). The column was washed with 5 ml sterile PBS, then 5000 neutralizing units of antiserum in 0.5 ml PBS was applied and the column was washed with 4.5 ml PBS. Then the bound material was eluted with 1 ml 1 M-acetic acid. The eluate was immediately neutralized with 2 ml 1 M-Na\(_2\)HPO\(_4\) and subsequently dialysed against PBS. The column separation was carried out at room temperature, with dialysis at 4°C.

**Incorporation of 5-[\(\text{H}\)]uridine.** Confluent monolayers of L929 cells in 24-well Falcon culture plates were used. At time zero the cells were washed once with MEM. One set of cells was infected with EMC virus (m.o.i. 50), whereas the other was not. Infected and uninfected cells were also exposed to 1 μCi 5-[\(\text{H}\)]uridine. Further additions to both sets of cells were PGA1 (10 μg/ml), actinomycin D (3 μg/ml), or both, the final volume of culture medium being 0.2 ml. Two, 4 and 6 h post-infection, supernatants of cells were removed and the monolayers washed once with 0.2 ml ice-cold PBS. Cells were then trypsinized (0.02% trypsin) and precipitated in 5 ml 5% aqueous TCA containing 20 μg/ml carrier DNA. Acid-insoluble radioactivity was determined by scintillation counting. All determinations were carried out in duplicate.

**Incorporation of [\(\text{S}\)]methionine.** Confluent monolayers of L929 cells in 24-well culture plates were washed twice with serum-free MEM lacking methionine. One set of cells was taken up in 200 μl methionine-free MEM, to which 6 μCi/ml [\(\text{S}\)]methionine was added. The other set, in addition, contained 6 μg/ml PGA1. After the cells had been kept at 37°C for the indicated times, media were removed and the monolayers washed twice with PBS. Cells were trypsinized and incorporated radioactivity was determined by TCA precipitation and scintillation counting. All determinations were carried out in duplicate.

**Indirect immunofluorescence.** L929 cells grown as a dense monolayer on round glass coverslips (1 cm diam.) were treated with interferon or PGA1, as indicated in the text, and infected with EMC virus at an m.o.i. of 50. After 5 h, cells were rinsed once with PBS, then fixed at \(-20°C\) by keeping them in methanol for 5 min followed by acetone for 2 min. Incubation with antibodies was performed in a humidified chamber at 37°C for 30 min each, using a 1:50 dilution of the first antibody (anti-EMC virus) and a 1:20 dilution of the second antibody (fluorescent anti-rabbit Ig). [\(\text{S}\)]methionine-labelled proteins. Confluent monolayers of L929 cells in 24-well culture plates were washed twice with 200 μl methionine-free MEM. All cells were infected with EMC virus (m.o.i. 50) at the same time, using either methionine-deficient MEM to which [\(\text{S}\)]methionine (50 μCi/ml) had been added, or
complete MEM without radioactive methionine. In addition, one-half of each set of cells received PGA\textsubscript{1} (8 µg/ml). After 1 h at 37 °C supernatants were removed from those cells which had received radioactive methionine. These cells were washed twice with MEM and then lysed by applying 2 times 15 µl sample buffer, according to Laemmli (1970). Ninety min later a second set of cells was pulse-labelled with [\textsuperscript{35}S]methionine for 1 h in the absence or the continued presence of 8 µg/ml PGA\textsubscript{1}, as described above, then likewise lysed with sample buffer. This process was repeated with a third set of cells from 4.5 to 5.5 h post-infection. All determinations were carried out in duplicate. Electrophoresis was performed essentially as described by Laemmli (1970) using 10% polyacrylamide discontinuous slab gels.

2',5'-Oligoadenylate synthetase assay. Cells were harvested by scraping, washed in cold PBS and stored at −70 °C as cell pellets. Frozen pellets were lysed in an NP40 buffer (0.5% NP40, 90 mM-potassium chloride, 1 mM-magnesium acetate, 10 mM-HEPES pH 7.6, 2 mM-2-mercaptoethanol) and post-mitochondrial supernatants were prepared. The Bio-Rad dye-binding assay was used for protein determination (Bradford, 1976). Externally corresponding to 100 µg protein were bound to poly(I)-poly(C) linked to cellulose (kindly provided by R. H. Silverman) and incubated for 2 h at 30 °C in buffer containing 10 mM-HEPES pH 7.6, 50 mM-potassium chloride, 15 mM-magnesium acetate, 7 mM-2-mercaptoethanol, 8 mM-ATP and 20% (v/v) glycerol (Stark et al., 1981). The 2',5'-oligoadenylate was measured in a radiobinding assay as described by Silverman et al. (1982).

Protein kinase assay. Cell extracts from NP40-lysed cells as described above were used. Protein (100 µg) bound to cellulose-linked poly(I)-poly(C) was incubated in the presence of [\textgamma-\textsuperscript{32}P]ATP as described by Krause et al. (1985), except that 3 µCi [\textgamma-\textsuperscript{32}P]ATP was added per assay.

RESULTS

Specificity and characteristics of the antiviral effect

Since antiviral effects of prostaglandins on EMC virus have not been previously described, we first established the parameters involved in the protection of L929 cells from this virus. As seen in Fig. 1, PGA\textsubscript{1} and PGA\textsubscript{2} were equally inhibitory to the propagation of EMC virus. Over 90% inhibition of virus yield was observed at concentrations of 4 µg/ml, similar to inhibitory concentrations seen with other viruses (Santoro et al., 1980, 1981, 1982a, 1983a). There was no difference in antiviral potency when PGAs were added together with the infecting virus as compared to adding them 1 h later (data not shown). In agreement with the results with Sendai virus (Santoro et al., 1981) no antiviral effect was seen when the cells were preincubated with PGA\textsubscript{1} for 5 h, then washed prior to infection and kept in the absence of PGA\textsubscript{1} for the remainder of the incubation period. Also, the antiviral effect was identical when PGA\textsubscript{1} was either added to cells together with infecting virus or 5 h prior to infection, but then kept with the infected cells for the entire incubation period. Antiviral activity was specific for prostaglandins of the A series. None of the others tested (B\textsubscript{1}, B\textsubscript{2}, D\textsubscript{2}, E\textsubscript{1}, E\textsubscript{2}, F\textsubscript{1a} or F\textsubscript{2a}) had any antiviral effects up to concentrations of 14 µg/ml, nor had they any effects on viability of L929 cells up to concentrations of 20 µg/ml, as determined by trypan blue dye exclusion after 7 h of cell exposure.

Under the conditions of the assay (7 h at 37 °C) PGAs lacked toxic effects on L929 cells at concentrations as high as 20 µg/ml, as determined by counting trypan blue dye-excluding cells. Cellular protein synthesis was unaffected after incubation of cells with 6 µg/ml PGA\textsubscript{1} for 8 h, a prostaglandin concentration that caused more than 90% inhibition of virus yield after 6 h incubation of the infected cells. Cellular RNA synthesis was also not inhibited by PGA\textsubscript{1}, as further discussed below (see Fig. 4). Thus, the antiviral action of PGAs is neither a consequence of effects on integrity nor on RNA or protein synthesis of the cells.

From the fact that PGAs are antiviral when added to previously infected cells, one can conclude that the antiviral effect is not due to inactivation of the infecting virus. As seen in Fig. addition of PGA\textsubscript{1} as late as 4 h post-infection yielded partial protection. On the other hand its presence for 2 h only, from hours 1 to 3 or 5 to 7 post-infection, was insufficient to cause virus inhibition. Since the presence of PGA\textsubscript{1} for 4 h, either from hours 1 to 5 or 3 to 7, yielded comparable protection, it appears that PGA\textsubscript{1} acts at a later stage of virus replication and is most effective when present during a period that includes hours 3 to 5 post-infection.

Independence of antiviral action of PGA from induction of cellular proteins

As suggested from the kinetics of the PGA effect, antiviral activity does not appear to involve intermittent interferon induction. This was directly verified using two approaches. In one we
assayed supernatants of PGA₁-preincubated L929 cells for the presence of interferon activity. Such supernatants were added to fresh sets of L929 cells in serial twofold dilutions and left on the cells for 24 h. After washing of the cells to remove residual PGA₁, they were infected with EMC virus as described for the standard interferon assay in Methods. No antiviral activity was detected in any of the original supernatants from cells exposed to concentrations between 2 and 10 µg/ml PGA₁ for 7, 20 or 30 h, respectively. This assay would have allowed detection of at least 5 IU/ml of interferon activity.

That interferon must start its action from the outside of the cell is well established. The presence of anti-interferon antibody in the medium of poly(I)·poly(C)-stimulated cells blocks the antiviral action of interferon produced by these cells (Vengris et al., 1975). Therefore, in a second approach we employed antiserum to mouse α/β interferon that had been partially purified by Protein A affinity column chromatography. As shown in Table 1, the antiviral activity of PGA₁ was not inhibited when purified anti-interferon antibody was present during the incubation period at a 60-fold dilution. The same antibody, diluted 1:300, completely blocked the antiviral activity of 100 IU/ml of mouse α/β interferon. It should be mentioned that the rabbit-derived crude antiserum as well as sera from unimmunized rabbits inhibited PGA₁ action at a 1000-fold dilution. However, this inhibition was clearly due to serum components.
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Table 1. Antibody to mouse α/β interferon does not block antiviral activity of PGA1

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<tr>
<th>Antiviral agent*</th>
<th>Dilution of antibody</th>
<th>Virus yield † (% control)</th>
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<tr>
<td>PGA1</td>
<td>No antibody</td>
<td>&lt;1</td>
</tr>
<tr>
<td>PGA1</td>
<td>1:60</td>
<td>&lt;1</td>
</tr>
<tr>
<td>α/β interferon</td>
<td>No antibody</td>
<td>&lt;1</td>
</tr>
<tr>
<td>α/β interferon</td>
<td>1:300</td>
<td>100</td>
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* Protein A affinity column-purified antibody was preincubated with PGA1 (8 μg/ml) or mouse α/β interferon (100 IU/ml) for 1 h prior to adding the mixtures to EMC virus-infected cells (in the PGA experiment) or to uninfected cells (in the interferon experiment).
† After 17 h incubation EMC virus yield was determined immediately in the case of the PGA-incubated cells. Interferon-treated cells were infected at that time and viral yield was determined 24 h later.

other than immunoglobulins, since Protein A affinity column chromatography separated the rabbit antiserum into unadsorbed material inhibitory to PGA1 action and without effect on α/β interferon (data not shown), and adsorbed and subsequently eluted material that inhibited interferon but not PGA1 action. Among various lots of foetal bovine serum, only one showed a small degree of inhibition of the antiviral effect of PGA1 at a 1:10 dilution. Horse and calf sera at that dilution were not inhibitory. To avoid any arbitrary effects of serum factors, therefore, all assays involving prostaglandins were carried out under essentially serum-free conditions as described in Methods.

To investigate whether the antiviral action of PGAs is the result of induction of other cellular proteins, the effect of PGA1 on EMC virus replication was determined in the presence of actinomycin D. This antibiotic blocks transcription of DNA into cellular RNA, but does not interfere with RNA-dependent viral RNA synthesis. Under the conditions of the standard assay, actinomycin D at concentrations up to 4 μg/ml did not interfere with the antiviral action of PGAs, strongly suggesting that induction of new cellular proteins is not essential for the antiviral effect of PGA1 (Fig. 3).

Two dsRNA-dependent enzymes, a protein kinase and a 2′,5′-oligoadenylate synthetase, are implicated in inhibition of virus replication in interferon-treated cells, and cellular levels of both enzyme activities are increased after interferon treatment (Baglioni, 1979). Although the results of the actinomycin experiment indirectly ruled out the possibility that induction of one or both of these enzymes is involved in the antiviral action of PGAs, we directly confirmed that conclusion by comparing both enzyme activities in extracts of PGA1-preincubated cells to those in extracts of control cells. As seen in Table 2, no measurable increase in synthetase activity was observed in extracts derived from cells treated with PGA for 5 or 17 h. As expected, and shown for comparison, interferon pretreatment of the cells yielded over 100-fold augmentation of this enzyme activity. Furthermore, no increase in protein kinase activity was apparent in extracts from PGA1-treated cultures, after incubation with 6 μg/ml for 5 or 24 h, as judged from SDS-PAGE of reaction products obtained in protein kinase assays using [γ-32P]ATP. No increase in radioactivity of the band migrating with an apparent molecular weight of 65000 was found when extracts from PGA1-treated cells were compared with those from control cells. Extracts from interferon-pretreated cells assayed identically clearly showed increased amounts of radioactivity associated with this band (data not shown).

Effects of PGA on viral RNA and protein synthesis

That PGA1 has no effect on cellular RNA synthesis under the conditions of the standard antiviral assay is shown in Fig. 4. There was no difference in [3H]uridine incorporation into TCA-precipitable counts in the absence or in the presence of PGA1. In this experiment RNA-associated radioactivity in control cells was lower after 6 h than at 4 h, presumably due to RNA turnover concomitant with decreasing 5-3H]uridine in the medium. However, that decrease was identical regardless of whether or not PGA1 was present. As expected, actinomycin D inhibited RNA synthesis in uninfected cells, whereas in EMC virus-infected cells viral RNA synthesis did proceed. Furthermore, viral RNA synthesis was strongly decreased in the presence of PGA1,
Fig. 3. Lack of effect of actinomycin D on the antiviral activity of PGA. Confluent monolayers of L929 cells were infected with EMC virus at an m.o.i. of 50 in the absence (○) or in the presence of actinomycin D (△, 2 μg/ml; ▽, 4 μg/ml). After 1 h at 37°C supernatants were removed and cells exposed to the indicated prostaglandin concentrations, in the absence or the continued presence of actinomycin D, for another 6 h, when virus yield was determined by haemagglutination. Virus titres in the absence of actinomycin D were identical to those obtained in its presence.

Fig. 4. Incorporation of 5-[3H]uridine into cellular and viral RNA in the absence and in the presence of actinomycin D and PGA. Monolayers of L929 cells in 24-well Falcon plates received in addition to 5-[3H]uridine, either 10 μg/ml PGA1, 3 μg/ml actinomycin D, or both. One set of cells remained uninfected; the other was infected with EMC virus at an m.o.i. of 50. At indicated times cells were washed and TCA-insoluble radioactivity was determined. Uninfected cells: ○, no addition; △, plus PGA1; ▽, plus actinomycin D; ▲, plus PGA1 and actinomycin D. Infected cells: ■, plus actinomycin D; ▽, plus PGA1 and actinomycin D.

Table 2. Lack of induction of 2',5'-oligoadenylate synthetase by PGA

<table>
<thead>
<tr>
<th>Cells preincubated with*</th>
<th>2',5'-Oligoadenylate synthetase activity (pmol oligo(A)/mg protein per h)</th>
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<tr>
<td>-</td>
<td>&lt;50</td>
</tr>
<tr>
<td>PGA1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>α/β interferon</td>
<td>6900</td>
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* Cells were preincubated for 17 h with either 10 μg/ml PGA1, or 500 IU/ml mouse α/β interferon. (After 5 h of incubation with 10 μg/ml PGA1, the synthetase was also not elevated.)

This effect was quite pronounced between 4 and 6 h, consistent with a block in virus maturation during this time period.

Similar inhibition was also apparent when viral antigen production in infected cells was visualized by indirect immunofluorescence (Fig. 5). When the infected cells were kept in the presence of 5 μg/ml PGA1 for 5 h (Fig. 5b), viral antigen in the cytosol was undetectable, as shown by the absence of brightly fluorescent antigen–antibody complexes that are visible throughout the cytoplasm of control cells treated identically in the absence of PGA1 (Fig. 5a). Even after longer incubation of infected cells with PGA1 (8 or 17 h) indirect immunofluorescence did not reveal virus-positive cells, indicating that protection by PGA1 is due to inhibition of virus replication and not to an extension of the viral replication cycle. Consistent with results described in Fig. 2, PGA1 was active in preventing immunofluorescence inside the cells when added up to 2 h after infection. Preincubation with mouse α/β interferon yielded a comparable inhibition of viral antigen–antibody complex formation 5 h post-infection, as shown in Fig. 5c. In contrast to PGA-treated cell monolayers, in this case some of the infected cells still synthesized viral antigen. This phenomenon was found to be reduced when the amounts of interferon used were increased, but lacks an explanation at the present time. These data again
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Fig. 5. EMC virus protection assay as revealed by indirect immunofluorescence. (a) Control cells without antiviral treatment 5 h post-infection. The arrowhead indicates an unstained (probably uninfected) cell surrounded by brightly fluorescent cells which carry the viral antigen throughout their cytoplasm. (b) Cells treated with 5 μg/ml PGA1 at the time of infection, stained by indirect immunofluorescence 5 h later. (c) Cells pretreated with 100 IU/ml mouse α/β interferon for 18 h prior to infection. Immunofluorescence was determined 5 h post-infection. All three experiments were run in parallel. Note the complete absence of brightly fluorescent cells in the case of PGA-treated cells (b), contrasting with the situation after interferon treatment which leaves a certain number of cells unprotected (c). Bar markers represent 50 μm.

suggest that PGA interferes with an event (or events) in the growth cycle of the virus that manifests itself during a period within 5 h post-infection, and thus must be independent of virus release.

We also determined whether PGA treatment preferentially inhibited synthesis and/or processing of one or only a few viral proteins, or whether the effect was a more general one affecting most or all viral proteins. In this experiment we compared newly synthesized proteins in EMC virus-infected cells, either untreated or treated with PGA1, after 1 h of pulse-labelling with [35S]methionine, by subjecting the labelled cell lysates to SDS-PAGE. As illustrated in Fig. 6, PGA1 had little effect on cellular protein synthesis during a 1 h pulse from 0 to 1 or from 2.5 to 3.5 h after infection. No labelled viral proteins were detectable on the autoradiograms for these labelling periods. A [35S]methionine pulse from 4.5 to 5.5 h post-infection revealed that all the major proteins commonly associated with EMC virus are labelled. The same pattern of radioactive proteins was found in PGA-treated cells, although the amount of radioactivity associated with each band was much reduced. Thus, PGA treatment seems to affect all the major viral proteins in a similar manner, and does not affect processing of viral proteins.

DISCUSSION

Our results confirm and extend previous reports of antiviral effects of prostaglandins of the A series (Santoro et al., 1980, 1981, 1982a, 1983a), adding EMC virus to the list of inhibitable viruses. The effect involves neither infection nor virus release, since PGA added during 1 to 3 h or during the last 2 h after infection was without consequence for the yield of new virus. Thus, the antiviral event must occur at some time between 3 and 5 h. EMC virus-infected L929 cells show a drastic decrease in the rate of cellular protein synthesis which reaches a minimum around 4 h post-infection. Subsequently, the rate of protein synthesis increases again rapidly to a maximum around 5.5 h after infection, representing the synthesis of viral proteins (Ramabhadran & Thach, 1981). Thus, events immediately preceding the onset of viral protein synthesis seem to be involved in the antiviral activity of PGAs. Clearly, the antiviral effect is
Fig. 6. Separation of cellular and viral proteins from L929 cells infected with EMC virus, after pulse-labelling with $[^{35}\text{S}]$methionine. Confluent monolayers of L929 cells in 24-well Falcon culture plates were infected with EMC virus and incubated with $[^{35}\text{S}]$methionine in methionine-deficient MEM for 1 h starting at (a) 0, (b) 2.5 and (c) 4.5 h, as described in Methods. Thereafter, labelled cells were lysed and subjected to electrophoresis. The dried gel was treated with En3Hance and subjected to autoradiography. The plus and minus signs on the top of the gel indicate whether or not PGA$_1$ (7 µg/ml) was present. Molecular weight markers are indicated at the left and were from top to bottom: rabbit muscle myosin, Escherichia coli β-galactosidase, rabbit muscle phosphorylase b, bovine serum albumin and ovalbumin. Arrows on the right indicate major viral proteins formed (Ramabhadran & Thach, 1981).

established by 5 h, since indirect immunofluorescence at this time reveals absence of viral antigens in the cytoplasm of infected cells (Fig. 5). As seen in Fig. 4 viral RNA starts to increase by 4 h and continues to rise up to about 6 h post-infection. It is that increase in viral RNA synthesis which is largely abolished in PGA-treated cells, strongly suggesting that this block in turn leads to inhibition of the synthesis of viral proteins.

A relationship between the antiviral actions of interferons and prostaglandins has been suggested by several laboratories (Santoro et al., 1983b; Yaron et al., 1977; Fitzpatrick & Stringfellow, 1980). Interferons might cause inhibition of virus by increasing cellular PGA levels, which then might be directly responsible for the antiviral effect or, indirectly, by causing subsequent induction of antiviral proteins (Santoro et al., 1982b, 1983b). A less likely explanation for the antiviral effect of PGAs might be that it is the result of intermittently produced interferon(s).

Our data directly exclude the possibility that interferons are induced in response to PGA
treatment. There was no detectable interferon activity in supernatants of PGA-treated cells. Furthermore, anti-mouse \( \alpha/\beta \) interferon antibody had no inhibitory effect on the antiviral activity of PGA, when applied in amounts that would have neutralized the activity of 500 IU/ml of that interferon in the medium.

It is curious that serum from non-immunized rabbits inhibits the antiviral effect of PGA, in contrast to foetal bovine, calf or horse sera. Polet & Levine (1975) have reported that rabbit serum contains a very potent PGA isomerase that rapidly converts prostaglandin A to prostaglandin C. This enzyme could account for the inhibitory effect of rabbit serum on the antiviral activity of PGAs. The observation that this enzyme is not detectable in horse or bovine sera correlates well with the fact that horse and calf sera are not inhibitory to the antiviral action of PGA.

Our data rule out the possibility that cellular proteins implicated in the antiviral action of interferons are induced in response to PGA treatment. The antiviral effect is unperturbed under conditions where cellular RNA synthesis is blocked by actinomycin D. Furthermore, we find no change in the levels of the two dsRNA-dependent enzymes, protein kinase and \( 2',5' \)-oligoadenylate synthetase, believed to be responsible for antiviral action of interferons (Baglioni, 1979). These data are at variance with those of Santoro et al. (1983b), who describe increased levels of both enzymes in cells incubated with PGA1. We have no explanation for this discrepancy since our experiments, as well as theirs, involved mouse L929 cells and PGA1 at comparable concentrations and incubation periods. Clearly, these two enzymes are not induced by interferons in the presence of actinomycin D, a condition that also prevents its antiviral action, yet has no consequence for the antiviral activity of PGA1 (Fig. 3).

Conceivably, antiviral activity of interferons could be mediated through increased levels of PGAs. Metabolic inhibitors of prostaglandin biosynthesis which block fatty acid cyclooxygenase, like indomethacin or aspirin, have been reported to inhibit the antiviral action of interferon (Pottathil et al., 1980). However, a lack of such effects on interferon action has been reported from other laboratories (Tovey et al., 1982; Milhaud et al., 1983). Mouse \( \alpha/\beta \) interferon-resistant cells (L-1210R) which lack a functional cyclooxygenase (Chandrabose et al., 1981), have also been shown to lack receptors for this class of interferons (Aguet, 1980). Thus, interferon resistance of L-1210R cells can be explained equally as well by the absence of interferon receptors as by the absence of cyclooxygenase activity. Increased concentrations of prostaglandins E and F2\(_{\alpha}\) in the medium of interferon-treated cells have been observed (Fitzpatrick & Stringfellow, 1980; Yaron et al., 1977; Santoro et al., 1983b; Fuse et al., 1982). As shown here and by others (Santoro et al., 1980, 1981, 1982a) neither of these prostaglandins has an antiviral activity against EMC, Sendai or vaccinia viruses. Also, L929 cells, after 24 or 48 h of treatment with 500 IU/ml mouse \( \alpha/\beta \) interferon, failed to produce increased PGA concentrations in the medium, as judged from measuring HPLC-separated fractions of solvent-extracted and silicic acid-purified samples with a radioimmune assay employing PGA-specific antibody (Santoro et al., 1983b). As stated by these authors, mouse L cells normally synthesize small amounts of immunoreactive PGA, over 90% of which is found in the medium in concentrations of 1 ng/ml or less, and increased to no more than 5 ng/ml in vaccinia virus-infected cells treated with interferon. Clearly, such concentrations of PGAs in the medium of EMC virus-infected L cells are without consequence for the proliferation of this virus, and also have no antiviral effects on Sendai, vaccinia and vesicular stomatitis viruses (Santoro et al., 1980, 1981, 1982a, 1983a).

Therefore, the hypothesis that the antiviral actions of PGAs and interferons share a common mechanism is, at least in the case of EMC virus, not supported by the available evidence. Rather it appears that these antiviral agents have distinct modes of action by which they prevent virus proliferation. Interferons require cellular RNA and protein syntheses for antiviral activity; PGAs, on the other hand, act independently from these processes. The precise mechanism of antiviral action of prostaglandins of the A series at the molecular level remains to be elucidated.

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