ELSEVIER

Antiviral Research 26 (1995) 83–96

Antiviral Research

Antiviral effect of cyclopentenone prostaglandins on vesicular stomatitis virus replication

Judy Parker *, Patricia B. Ahrens, Helmut Ankel

Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226 USA

Received 3 August 1994; accepted 27 September 1994

Abstract

Prostaglandins are potentially useful antiviral agents, however their mechanism of action is unclear. Recent evidence suggests that RNA transcription of vesicular stomatitis virus (VSV) is inhibited by prostaglandins (Bader and Ankel, J. Gen. Virol. 71, 2823–2832, 1990). Prostaglandins are known to have multiple effects on cells which may or may not be related to their antiviral action. We examined the effects of prostaglandins on cells and on VSV RNA polymerase in vitro to seek the mechanism of antiviral action. Actinomycin D inhibited cellular RNA synthesis but failed to block the antiviral activity of prostaglandins on VSV. Thus induction of host cell RNA transcription is not involved in the antiviral action. Neither modulation of the cellular glutathione level by prostaglandins nor formation of prostaglandin-glutathione conjugates was required for the antiviral action. The relative inhibition of VSV RNA polymerase in vitro by prostaglandins with different structures correlated to inhibition of VSV replication in infected cells. This result indicates that the same step in VSV replication is inhibited by prostaglandins both in the in vitro RNA polymerase assay and in the infected cell.

Keywords: Prostaglandin A_2; Antiviral compound; Glutathione conjugate; Vesicular stomatitis virus

1. Introduction

Prostaglandins have been found to effectively inhibit the replication of several types of viruses (reviewed in Santoro et al., 1990; Pica et al., 1993). The antiviral mechanism(s) is not well understood and may be different for different viruses. Recent evidence indicates that prostaglandins decrease both the viral mRNAs and proteins in cells

* Corresponding author. Present address: Department of Internal Medicine, Box 70,622, East Tennessee State University, Johnson City, TN 37614-0622. Fax: +1 (615) 9296387.

SSDI 0166-3542(94)00067-0

infected with vesicular stomatitis virus (VSV) (Bader and Ankel, 1990; Bader et al., 1991). Others have also reported that viral proteins of VSV are decreased or not made in the presence of prostaglandins (Pica et al., 1993). The decrease in viral mRNAs is probably the source of the decrease in viral proteins, however the mechanism through which prostaglandins decrease the viral mRNAs of VSV is not clear. It is possible that prostaglandins directly inhibit the viral RNA polymerase activity in infected cells, since VSV RNA polymerase is inhibited by relatively high concentrations of prostaglandin A_1 in vitro (Bader and Ankel, 1990; Bader et al., 1991)

The most potent antiviral prostaglandins contain a cyclopentenone group and it has been suggested that true antiviral activity is restricted to only these prostaglandins (Yamamoto et al., 1987). Cyclopentenone prostaglandins have multiple effects on cells, and some of these effects might be involved in the antiviral action. Cyclopentenone prostaglandins are known to induce the synthesis of heat shock proteins (Ohno et al., 1988; Santoro et al., 1989), hemeoxygenase (Koizumi et al., 1992a), and γ-glutamylcysteinyl synthetase (Ohno and Hirata, 1990). The induction of at least the heat shock proteins and γ-glutamylcysteinyl synthetase occurs at the level of RNA transcription since actinomycin D inhibits the induction of these proteins by prostaglandins (Ohno et al., 1988; Ohno and Hirata, 1990). Furthermore, it is known that prostaglandins induce the synthesis of heat shock proteins through activation of the heat shock factor which regulates transcription from the heat shock genes (Amici et al., 1992; Holbrook et al., 1992). The induction of heat shock proteins is associated with the antiviral action of prostaglandins against Sindbis virus (Mastromarino et al., 1993) and perhaps against Sendai virus as well (Amici and Santoro, 1991). However, induction of cellular RNA transcription is not required for the antiviral action of prostaglandins on encephalomyocarditis virus (Ankel et al., 1985). Thus whether the induction of cellular RNA transcription is required for the antiviral effect of prostaglandins must be determined for each type of virus.

The uptake and metabolism of cyclopentenone prostaglandins may be related to the antiviral action(s). Recently, it was found that cyclopentenone prostaglandins are rapidly metabolized by cells into prostaglandin-glutathione conjugates (Atsmon et al., 1990a, Atsmon et al., 1990b; Parker and Ankel, 1992). Large amounts of PGA_2-glutathione conjugates form and accumulate inside L1210 cells eventually depleting the cellular glutathione (Parker and Ankel, 1992). Furthermore, the effect of prostaglandins on glutathione may be related to the induction of heat shock proteins and hemeoxygenase (Koizumi et al., 1992b). Whether the metabolism of cyclopentenone prostaglandins by cells is involved in the antiviral action of the prostaglandins has not yet been evaluated. Here we have examined several prostaglandin effects to determine which are required for the antiviral action against VSV.

2. Materials and methods

2.1. Chemicals

We obtained Eagle's minimal essential medium (MEM), RPMI-1640, Ham's F12 nutrient mixture, Dulbecco's phosphate-buffered saline, Hank's balanced salt solution,

fetal calf serum, and horse serum from GIBCO BRL, Gaithersburg, MD. The Triton X-100 was from Bio-Rad Laboratories, Hercules, CA. Prostaglandin J_2 and 13,14-dihydro-15-keto-prostaglandin A_2 were from Cayman Chemical, Ann Arbor, MI. The 15-epi-prostaglandin A_2 was a gift from Upjohn Co., Kalamazoo, MI. The other prostaglandins and chemicals were purchased from Sigma Chemical Co, St. Louis, MO.

2.2. Cell lines and cell culture

The cell lines were obtained from the American Type Culture Collection: L1210 (CCL219), L929 (CCL1), BHK (CCL10) and MDBK (CCL22). The L1210 cells were routinely cultured in RPMI-1640 medium supplemented with 10% horse serum. The L929 cells were grown in minimal essential medium (MEM) supplemented with 10% fetal calf serum. The MDBK and BHK cells were grown in plastic flasks in Ham's F12 nutrient mixture supplemented with 10% fetal calf serum. Cells growing exponentially were used to initiate all experiments.

2.3. Assays to determine antiviral action

L929 cells were seeded at 5×10^4/well in MEM, 10% fetal calf serum in a 96 well plate (Costar) and incubated overnight at 37°C. The medium was then replaced with VSV at a multiplicity of infection (MOI) = 20 for 1 h. The wells were washed 3 times with phosphate-buffered saline (PBS) and 0.1 ml of MEM, 25 mM Hepes containing various concentrations of antiviral compounds was added to all wells in triplicate. After 6 h at 37°C the plates were frozen at −70°C and the VSV yield was quantified by a miniplaque assay in MBDK cells (Langford, et al., 1981). L929 cells were infected with EMC virus as for VSV; however, the yield of EMC was determined by hemagglutination assay (Jameson et al., 1977).

L1210 cells (3×10^6/ml) were infected with VSV at MOI = 20 for 1 h at 37°C. The cells were washed 3 times with RPMI-1640, 25 mM Hepes and diluted to 1×10^6/ml in the same medium. Then 0.1 ml of cells was mixed with 0.1 ml of RPMI-1640, 25 mM Hepes containing various concentrations of prostaglandins in 96 well plates. After 6 or 11 h at 37°C, the plates were frozen until VSV yield was determined by miniplaque assay as above.

Some experiments used glutathione-depleted L1210 or L929 cells in the antiviral assays. In this case, cells were cultured for 16 (L929) or 20 (L1210) h in buthionine sulfoximine before infection with viruses. The buthionine sulfoximine was maintained at the same concentration during infection and the subsequent addition of antiviral compounds.

Experiments with L929 cells and actinomycin D were performed as above except that some wells received 1 μCi of [^{3}H]uridine at the time of addition of prostaglandin. Actinomycin D stock solution was prepared in dimethylsulfoxide and the final concentration of dimethylsulfoxide in the medium (0.1%) did not inhibit replication of VSV. After 6 h the incorporation of [^{3}H]uridine into RNA was quantitated by precipitation in 20% trichloroacetic acid, harvesting onto glass fiber filters, and counting in scintillation fluid.

2.4. Determination of cellular glutathione content

L929 cells were seeded in culture medium in 12 well plates (5×10^5/well) and incubated overnight at 37°C. The medium was removed and the plates were washed 3 times with PBS. Then 1 ml of MEM, 25 mM *N*-[2-hydroxyethyl]piperazine-*N'*-[2-hydroxypropane]sulfonic acid (Hepes) which contained PGA_2 or not, was added to all wells for the time indicated. Medium was then decanted and 0.3 ml of 0.25% trypsin in Hank's balanced salt solution was added for 5 min to release the cells from the plastic. The cells were then transferred into tubes, centrifuged, and the pelleted cells solubilized in 30 μl of 1% Triton X-100, 38 μl of 10% 5-sulfosalicylic acid and 50 μl of H_2O. The precipitate was removed by centrifugation at $10\,000 \times g$ for 10 min and the clear supernatant was assayed for total glutathione with glutathione reductase as described (Parker and Ankel, 1992). Aliquots of L1210 cells were centrifuged and the pelleted cells were treated as above.

2.5. VSV growth and purification

$VSV_{(Ind)}$ for antiviral assays was routinely grown at low multiplicity of infection (MOI) in L929 cells. $VSV_{(Ind)}$ for transcription assays was grown in BHK21 or MDBK cells at MOI = 0.1 in MEM, 2% fetal calf serum for 24 h. The medium from the infected cultures was centrifuged 15 min at $1300 \times g$ at 4°C and the supernatant then centrifuged at $100\,000 \times g$ for 2 h at 4°C. The VSV was then purified by centrifugation into a 0–40% (w/v) sucrose gradient in 10 mM Tris-HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA) at $35\,000 \times g$ for 90 min at 4°C. The lower virus band was diluted in Buffer A (10 mM Tris-HCl, pH 8.0, 0.4 M NaCl, 0.6 mM dithiothreitol) and centrifuged at $100\,00 \times g$ for 2 h at 4°C. The VSV pellet was then resuspended in 10 mM Tris-HCl pH 8, 1 mM EDTA and frozen at −70°C.

2.6. In Vitro assay of VSV RNA polymerase Activity

VSV RNA polymerase activity was determined as previously described (Baltimore et al., 1970; Banerjee et al., 1974). Purified VSV (approximately 0.4 mg/ml protein) was disrupted with an equal volume of 0.5% NP40 and then 0.75 volume of H_2O was added. Reaction mixtures contained 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.1 M NaCl, 350 units of RNasin, 0.05 mM CTP (including 10 μCi of [α^{32}P]CTP), 1 mM each of ATP, UTP, and GTP, and 19 μl of disrupted VSV in a total volume of 50 μl. In some reaction mixtures the above NTPs were replaced with 4 μCi [^{3}H]UTP, 0.05 mM UTP, and 1 mM each of ATP, CTP, and GTP. Prostaglandins (from stocks in ethanol) were added to a final concentration of 0.5 mM and equivalent ethanol was added to control samples. After 60 min at 30°C, two aliquots of 5 μl were removed and streaked onto Whatman DE81 filters. The filters were air dried, washed 5 times in 0.5 M Na_2HPO_4, once in H_2O, and rinsed in chilled ethanol. Filters were then counted for radioactivity in BioSafe II scintillation cocktail. Filter background (determined in reactions lacking VSV) was subtracted from all samples. Duplicate values were averaged (error was $< 5\%$ between duplicates) and the % of control calculated.

3. Results

3.1. Induction of host cell RNA transcription is not required for the antiviral effect of PGA_2

Since cyclopentenone prostaglandins are known to induce transcription of several cellular proteins, we determined whether the induction of cellular mRNA synthesis was involved in the antiviral action of prostaglandins. We used actinomycin D to inhibit cellular mRNA synthesis without inhibiting the replication of vesicular stomatitis virus (VSV). In uninfected cells [^{3}H]uridine incorporation into RNA was strongly inhibited by 5 μg/ml actinomycin D (Fig. 1). In the presence of actinomycin D, the residual RNA synthesis was greater in VSV infected cells than in the uninfected controls (Fig. 1). VSV RNA synthesis and replication is not inhibited by actinomycin D. Thus, synthesis of viral RNA should increase the actinomycin D resistant fraction in the infected cells. As would be expected, PGA_1 reduced the actinomycin D resistant RNA synthesis at concentrations which inhibit VSV replication (Fig. 1, Fig. 2). Clearly, actinomycin D did not prevent the antiviral action of PGA_1 against VSV in the L929 cells (Fig. 2). In addition, L1210 cells pretreated for 15 minutes with actinomycin D are also fully capable of an antiviral response to PGA_2 (not shown). These results indicate that the inhibition of VSV replication by PGA_1 or PGA_2 is independent of the induction of cellular proteins.

3.2. PGA_2-glutathione conjugates have no role in the antiviral action of PGA_2

Prostaglandins containing an α,β-unsaturated ketone (cyclopentenone) group are the most potent, and perhaps only, antiviral prostaglandins. Cyclopentenone prostaglandins are also the only prostaglandins able to form conjugates with glutathione (Atsmon et al.,

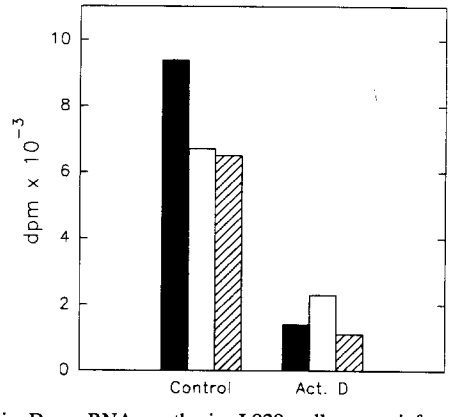

Fig. 1. Effect of actinomycin D on RNA synthesis. L929 cells were infected with VSV for 1 h. Then actinomycin D (5 μg/ml), PGA_1 (12 μM), and [^{3}H]uridine were added. After 5 hr at 37°C, the amount of radioactivity incorporated into RNA was determined as in Materials and methods. Closed bar, uninfected controls; solid bar, VSV infected; hatched bar, VSV + PGA_1.

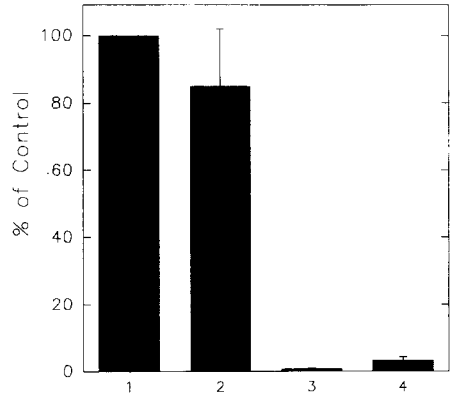

Fig. 2. Actinomycin D fails to block PGA inhibition of VSV replication. L929 cells were infected with VSV for 1 h. Then actinomycin D (5 μg/ml) and PGA_1 (12 μM) were added together. After 6 h the VSV produced from the duplicate wells was determined by miniplaque assay. Results presented as % of control are the mean $\pm$ S.E.M. for three experiments. The average control VSV titer was 3.6×10^5 PFU/ml. 1) Control, 2) Actinomycin D, 3) PGA_1, and 4) Actinomycin D and PGA_1.

1990a; Atsmon et al., 1990b; Parker and Ankel, 1992). Large amounts of prostaglandin-glutathione conjugates accumulate inside cells exposed to cyclopentenone prostaglandins and thus might be the active form of these prostaglandins. Previous studies with the L1210 cells have shown that few PGA_2-glutathione conjugates ($\leq 2\%$ of the control amount) are produced after depletion of glutathione by buthionine sulfoximine (Parker and Ankel, 1992). [Buthionine sulfoximine specifically inhibits the first enzyme in the pathway of glutathione synthesis (Meister, 1988)]. In both L1210 and L929 cells, buthionine sulfoximine reduces cellular glutathione to $< 2\%$ of the control levels without apparent toxicity (Table 1). Yet PGA_2 inhibits the replication of VSV in the cells depleted of glutathione as well as in control cells (Fig. 3). PGA_2 is actually less toxic in glutathione depleted cells (Parker and Ankel, 1992). Thus, the antiviral effect of PGA_2 in the glutathione depleted cells is not an artifact of enhanced toxicity. Clearly, the formation of PGA_2-glutathione conjugates is not required for the antiviral action of PGA_2.

3.3. Modulation of cellular glutathione by PGA_2 is not involved in the antiviral effect

Cellular glutathione is depleted in the L1210 cells as a result of the formation of PGA_2-glutathione conjugates (Parker and Ankel, 1992). An adequate level of cellular glutathione could be required for VSV to replicate inside a host cell, since it is known that reducing agents are required to preserve the VSV RNA polymerase activity in vitro (Massey and Lenard, 1987). Depletion of cellular glutathione by buthionine sulfoximine only marginally decreased VSV growth (Fig. 3), indicating that glutathione per se is not required for VSV growth. However, other protective thiols which substitute for glutathione are probably induced during the long-term buthionine sulfoximine pretreatment. Cyclopentenone prostaglandins might then inhibit VSV replication by depletion of the

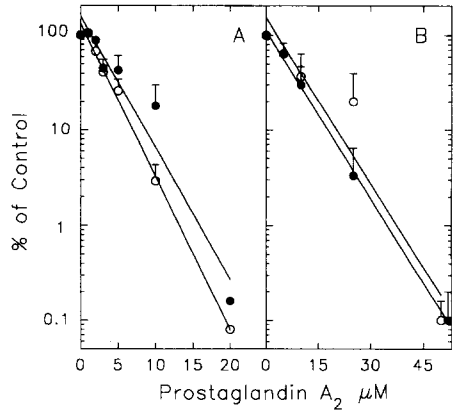

Fig. 3. PGA_2 antiviral action on VSV is independent of the formation of PGA_2-glutathione conjugates. (A) L1210 cells were cultured for 20 h in 1 mM buthionine sulfoximine, RPMI 1640, 10% horse serum to deplete glutathione. Control cells were cultured without the buthionine sulfoximine. Cells were infected with VSV followed by addition of various concentrations of PGA_2 in triplicate. After 7 h virus yield was determined by miniplaque assay and the % of control calculated for each triplicate. Results from three experiments are presented as the mean ± S.E.M. where each experiment provided its own control. The average VSV titer without PGA_2 was 8.4×10^6 PFU/ml in controls and 4.5×10^6 PFU/ml in BSO-treated. O-O, Control; ●-●, buthionine sulfoximine. (B) L929 cells were cultured for 16 h in 50 μM buthionine sulfoximine in MEM, 10% fetal calf serum to deplete glutathione. Control cells were cultured without the buthionine sulfoximine. The cells were then infected with VSV followed by addition of various concentrations of PGA_2 in triplicate. After 7 h the virus produced was determined by miniplaque assay and the % of control calculated for each triplicate. Results from three experiments are presented as the mean ± S.E.M., where each experiment provided its own control. The average titer without PGA_2 was 9×10^6 PFU/ml in controls and 11×10^6 PFU/ml in BSO-treated. O-O, Control; ●-●, Buthionine sulfoximine.

major protective thiol: whether glutathione or a substitute induced when glutathione is depleted. Therefore, we compared the concentration dependence of the glutathione loss with the antiviral action of PGA_2. A higher concentration of PGA_2 was required to decrease cellular glutathione than for inhibition of the replication of VSV in the L929 cells (Fig. 4). The same result was observed for the inhibition of EMC virus in L929 cells (Fig. 4). Although VSV-infected L929 cells contained less glutathione in the absence of PGA_2, the infected and uninfected L929 cells showed a nearly pattern of modulation of glutathione in response to PGA_2 (Fig. 4A). The slight increase in glutathione at low concentrations of PGA_2 was reproducible and may result from the induction of γ-glutamylcysteine synthetase activity which occurs in response to PGA_2 (Ohno and Hirata, 1990). It is evident that PGA_2 increases cellular glutathione at ≤ 6 μM and decreases cellular glutathione at ≥ 10 μM. The antiviral effect occurs at ≤ 6 μM and therefore does not depend on the depletion of glutathione.

3.4. Inhibition of VSV RNA polymerase by prostaglandins in vitro correlates with their antiviral action in infected cells

Previous results suggested that prostaglandins might inhibit the RNA polymerase of VSV (Bader and Ankel, 1990) but additional evidence is required to confirm this

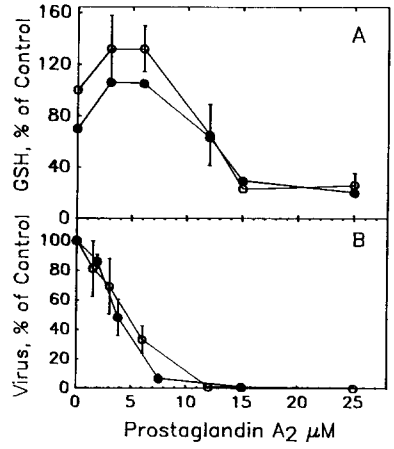

Fig. 4. Concentration dependence of glutathione loss and antiviral activity caused by PGA_2. L929 cells were infected or mock infected with VSV or EMC for 1 h. Various concentrations of PGA_2 were added for 6 h more and then virus yield and glutathione content were determined as in Materials and methods. The average VSV titer was 3.6×10^6 PFU/ml. (A) Cellular glutathione content as % of control: O-O, uninfected cells (mean ± S.D. $n = 4$); ●-●, VSV infected cells (average of two experiments). (B) Virus produced: O-O, VSV (three experiments, aver. ± S.E.M.); ●-●, EMC (four experiments, mean ± S.E.M.).

hypothesis. We compared the relative ability of various prostaglandins (Fig. 5) to inhibit VSV RNA polymerase in vitro and VSV replication in infected cells. In order to restrict our analysis to the earliest effect of prostaglandins, we halted all antiviral assays at 7 h post infection. At this time much viral RNA synthesis has occurred and significant amounts of complete VSV particles have been produced. Furthermore, the inhibition of VSV replication by prostaglandins at 7 h is maintained through the full virus cycle (Bader and Ankel, 1990; Santoro et al., 1983). The order of potency for inhibition in the RNA polymerase assay is $PGJ_2 = 15epiPGA_2 \geq PGA_2 >$ 13,14-dihydro-15-keto-$PGA_2 > PGB_2 = PGE_2$ (Fig. 6).

The order of potency for inhibition of VSV replication in L1210 cells is $PGJ_2 = 15epiPGA_2 = PGA_2 \gg PGB_2 =$ 13,14-dihydro-15-keto-$PGA_2 > PGE_2$ (Table 2). The relative order of potency in both assays is the same except for PGB_2 which is more potent than 13,14-dihydro-15-keto-PGA_2 in the antiviral assay but less potent than 13,14-dihydro-15-keto-PGA_2 in the RNA polymerase assay. PGB_2 can isomerize to PGA_2. It is therefore possible that more PGA_2 is generated from PGB_2 during the 6 h virus replication assay then is generated during the shorter in vitro RNA polymerase assay. These results suggest that the same mechanism is responsible for the prostaglandin inhibition both in the infected cell and in vitro.

4. Discussion

We find that induction of cellular mRNA transcription is not required for an antiviral effect of PGA_2 against VSV. This result is in agreement with evidence that the antiviral

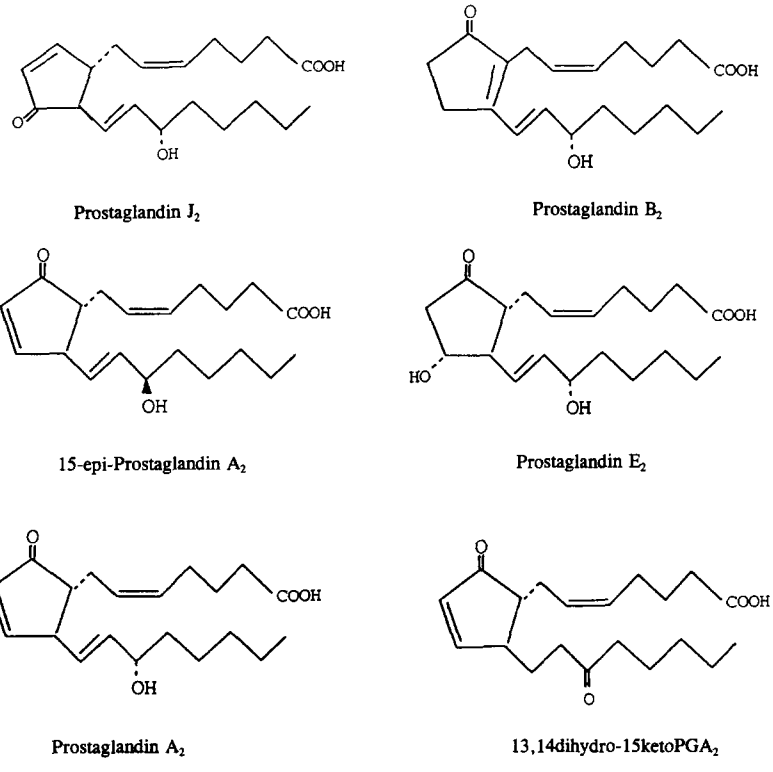

Fig. 5. Structures of prostaglandins.

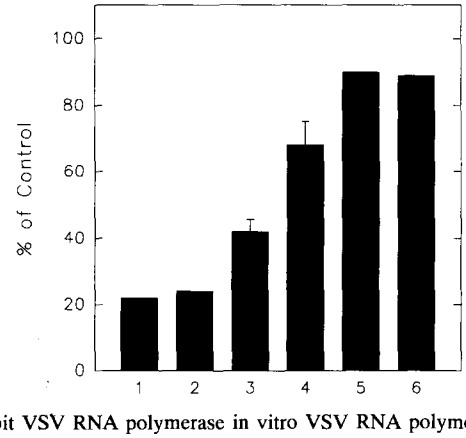

Fig. 6. Prostaglandins inhibit VSV RNA polymerase in vitro VSV RNA polymerase activity was assayed as described in Materials and methods. Various compounds were added at 0.5 mM and synthesis of RNA polymer was quantitated after 60 min at 30°C. Results are presented as % of control [control samples had 0.5% ethanol only]. Mean ± S.E.M. where available, others are average of duplicate samples which differed by less than 10%. 1 = PGJ_2, 2 = 15-epiPGA_2, 3 = PGA_2, 4 = 13,14-dihydro-15-keto-PGA_2, 5 = PGB_2, 6 = PGE_2.

Table 1
Effect of buthionine sulfoximine on cellular glutathione

Cell Type	Glutathione (nmol/10^6 cells)	
	Control	+ BSO #
L1210 Experiment 1	0.55	0.013
Experiment 2	3.4	0.1
Experiment 3	3.3	0.02
L929 Experiment 1	16.3	0.47
Experiment 2	11.4	0.11
Experiment 3	11.0	0.26

Cells were treated with buthionine sulfoximine, followed by determination of cellular glutathione content as described in Materials and methods.
Buthionine sulfoximine.

action of prostaglandins against encephalomyocarditis virus is also independent of cellular RNA transcription (Ankel et al., 1985). Heat shock proteins are induced by prostaglandins in VSV infected kidney cells (Pica et al., 1993). Strong evidence indicates that the induction of heat shock proteins by prostaglandins occurs through enhanced RNA transcription of the heat shock genes (Ohno et al., 1988; Amici et al., 1992; Holbrook et al., 1992). Since we find that the prostaglandin antiviral action against VSV is independent of cellular RNA transcription the induction of heat shock proteins by prostaglandins may occur in infected cells but is not involved in the antiviral action against VSV. In contrast, actinomycin D clearly prevents the antiviral effect of prostaglandins on Sindbis virus (Mastromarino et al., 1993). The ability of actinomycin D to prevent prostaglandin antiviral actions against Sindbis virus but not against VSV or EMC is probably due to differences between the viruses, perhaps related to the ability of the Sindbis genome to function as mRNA. Our results indicate that for VSV the cyclopentenone prostaglandins exert a more direct antiviral effect compatible with the hypothesis that prostaglandins inhibit virus specific RNA synthesis (Bader and Ankel, 1990).

Table 2
Inhibition of vesicular stomatitis virus replication by prostaglandins

Prostaglandin	50% Inhibition of VSV yield (mean ± S.E.M.)
PGA_2	3.0 ± 0.5 μM (14) *
PGJ_2	2 μM (1)
15epiPGA_2	2.3 ± 0.3 μM (3)
PGB_2	13.6 ± 2.2 μM (4)
13,14-Dihydro-15-ketoPGA_2	17.5 ± 3.4 μM (3)
PGE_2	33.2 ± 1.9 μM (5)

L1210 cells were infected with VSV and exposed to prostaglandins for 6 h as described in Materials and methods. VSV yield was determined by miniplaque assay.
* Number of experiments.

The vast majority of the PGA_2 within the cell is conjugated to glutathione (Parker and Ankel, 1992). Nonetheless, formation of PGA_2-glutathione conjugates is not required for the antiviral activity. Furthermore, depletion of cellular glutathione by prostaglandins required higher concentrations than for the antiviral action of prostaglandins. Thus, depletion of glutathione (or other protective thiol induced in the absence of glutathione) does not relate to the antiviral action. A slight *increase* in cellular glutathione content was observed in response to PGA_2 in the concentration range of the antiviral effects. An increase in cellular glutathione has previously been suggested to be antiviral: an elevation of cellular glutathione inhibits the induction of latent human immunodeficiency virus by various agents (Kalebic et al., 1991). However, the inhibition of VSV replication by PGA_2 cannot require an increase in glutathione synthesis, since PGA_2 is fully antiviral when glutathione synthesis is blocked by the presence of buthionine sulfoximine. The difference in these results is likely to be due either to differences between acute and latent viral infections or between the viruses studied.

Our results indicate that certain parts of the prostaglandin structure influence the antiviral potency. The cyclopentenone group was associated with highest potency. Previous results with PGA_1 (Bader and Ankel, 1990) are very similar to the our results with PGA_2. Interestingly, PGA_2 was similar to $\Delta^{12}PGJ_2$ in both assays of antiviral effect on VSV. Thus, the position of the keto group and the double bond in the pentene ring can be varied without a change in activity. 15-keto-PGA_2 was less potent than PGA_2 or 15-epiPGA_2 in both antiviral assays which indicates that the presence of a 15-OH is also important for high potency. PGE_2 has no cyclopentenone group and was required at 10-fold higher concentration than PGA_2 for antiviral activity in infected cells. It is known that PGE_2 can dehydrate to PGA_2 (Fitzpatrick and Wynalda, 1981; Polet and Levine, 1975; Stehle and Oesterling, 1977) leading to the hypothesis that only cyclopentenone prostaglandins are antiviral (Yamamoto et al., 1987). Yet PGE_2 and PGD_2 are antiviral although at higher concentrations compared to the cyclopentenone prostaglandins (Yamamoto et al., 1987; Bader and Ankel, 1990; Luczak et al., 1975; Giron, 1982). Our PGE_2 stocks did contain small amounts of PGA_2 upon HPLC analysis. The small amounts of PGA_2 present in the stock solution and generated during the experiment are probably responsible for the antiviral activity of PGE_2 against VSV in infected cells. This interpretation of our results is supported by the fact that PGE_2 does not inhibit VSV RNA polymerase activity in vitro even at concentrations which are 10 times the inhibitory concentration of PGA_2 (Bader and Ankel, 1990).

The relative ability of various prostaglandins to inhibit VSV replication in infected cells correlated to their inhibition of VSV RNA polymerase activity in vitro. This result suggests that the mechanism of inhibition of VSV RNA polymerase in vitro is the same as the antiviral mechanism in infected cells. Inhibition of viral RNA synthesis would provide an explanation for the decrease in VSV mRNAs observed in cells treated with prostaglandins (Bader and Ankel, 1990; Bader et al., 1991). However the concentration of prostaglandins required to inhibit VSV RNA polymerase activity in vitro is higher than is observed inside prostaglandin treated cells (Parker and Ankel, 1992). We find that PGA_2 binds to the VSV proteins in the detergent treated virus used in the RNA polymerase assay, but this does not appear to occur in infected cells (Parker, J., Ahrens,

P.A. and Ankel, H., unpublished results). If prostaglandins are depleted through binding to denatured VSV proteins then more prostaglandin would be required to achieve an effective free prostaglandin concentrations in vitro. Alternatively, other differences between RNA synthesis in detergent solubilized virus and in the infected cells may account for the puzzling concentration difference.

RNA polymerase of VSV requires the viral proteins L and P (NS) (Emerson and Yu, 1975). The L protein is the catalytic enzyme while the P protein is a regulatory phosphoprotein. Cellular protein kinases are present in the complete virions (Imblum and Wagner, 1974; Harmon et al., 1983). Furthermore, it is now known that a cellular protein kinase is required to phosphorylate the P protein as a requirement for RNA polymerase activity (Barik and Banerjee, 1992a, Takacs et al., 1992). This kinase is apparently casein kinase II (Barik and Banerjee, 1992b). The pp60src tyrosine kinase has also been found in complete VSV virions (Clinton et al., 1982) but it is uncertain if this kinase has a role in VSV replication. Prostaglandins could inhibit RNA polymerase activity through inhibition of the L protein or P protein function(s) or perhaps through inhibition of the cellular protein kinase(s) involved in VSV replication. Nonetheless, the requirement for 50–100-fold more prostaglandin to inhibit RNA polymerase in vitro then is required for inhibition in infected cells is difficult to understand. This result might be evidence that the in vitro inhibition of VSV RNA polymerase is unrelated to inhibition of VSV replication in infected cells. Further work will be required to determine the details of how prostaglandins inhibit VSV replication.

Acknowledgements

We thank Michael Kordus for excellent technical assistance. We also thank Dr. R.A. Johnson from the Upjohn Co. for the generous gift of 15-epiPGA$_2$. This work was supported by a grant from the National Institutes of Health, AI23450 and in part by a grant from the R.D. and Linda Peters Endowment to the Medical College of Wisconsin.

References

Amici, C. and Santoro, M.G. (1991) Suppression of virus replication by prostaglandin A is associated with heat shock protein synthesis. J. Virol. 72, 1877–1885.

Amici, C., Sistonen, L, Santoro, M.G., and Morimoto, R.I. (1992) Antiproliferative prostaglandins activate heat shock transcription factor. Proc. Natl. Acad. Sci. U.S.A. 89, 6227–6231.

Ankel, H., Mittnacht, S., and Jacobsen, H. (1985) Antiviral activity of prostaglandin A on encephalomyocarditis virus-infected cells: a unique effect unrelated to interferon. J. Gen. Virol. 66, 2355–2364.

Atsmon, J., Freeman, M.L., Meredith, M.J., Sweetman, B.J. and Roberts, L.J., II (1990a) Conjugation of 9-deoxy-Δ^9, Δ^{12} (E) prostaglandin D_2 with intracellular glutathione and enhancement of its antiproliferative activity by glutathione depletion. Cancer Res. 50, 1879–1885.

Atsmon, J., Sweetman, B.J., Baertschi, S.W., Harris, T.M. and Roberts, L.J., II (1990b) Formation of thiol conjugates of 9-deoxy-Δ^9 Δ^{12}prostaglandin D_2 and Δ^{12}(E)-prostaglandin D_2. Biochemistry 29, 3760–3765.

Bader, T. and Ankel, H. (1990) Inhibition of primary transcription of vesicular stomatitis virus by prostaglandin A_1. J. Gen. Virol. 71, 2823–2832.

Bader, T., Yamada, Y. and Ankel, H. (1991) Antiviral activity of the prostanoid clavulone II against vesicular stomatitis virus. Antiviral Res. 16, 341–355.

Baltimore, D., Huang, A.S. and Stampfer, M. (1970) Ribonucleic acid synthesis of vesicular stomatitus virus, II. an RNA polymerase in the virion. Proc. Natl. Acad. Sci. USA 66, 572–576.

Banerjee, A.K., Moyer, S.A. and Rhodes, D.P. (1974) Studies on the in vitro adenylation of RNA by vesicular stomatitus virus. Virology 61, 547-558.

Barik, S. and Banerjee, A.K. (1992a) Sequential phosphorylation of the phosphoprotein of vesicular stomatitis virus by cellular and viral protein kinases is essential for transcription activation. J. Virol. 66, 1109–1118.

Barik, S. and Banerjee, A.K. (1992b) Phosphorylation by cellular casein kinase II is essential for transcriptional activity of vesiculat stomatitis virus phosphoprotein P. Proc. Natl. Acad. Sci. USA 89, 6570–6574.

Clinton, G.M., Guerina, N.G., Guo, H. and Huang, A.S. (1982) Host-dependent phosphorylation and kinase activity associated with vesicular stomatitis virus. J. Biol. Chem. 257, 3313–3319.

Emerson, S.U. and Yu, Y.H. (1975) Both NS and L proteins are required for in vitro RNA synthesis of vesicular stomatitis virus. J. Virol. 15, 1348–1356.

Fitzpatrick, F.A. and Wynalda, M.A. (1981) Albumin-lipid interactions: prostaglandin stability as a probe for characterizing binding sites on vertebrate albumins. Biochemistry 20, 6129–6134.

Giron, D.J. (1982) Inhibition of viral replication in cell cultures treated with prostaglandin E_1. Proc. Soc. Exp. Biol. Med. 170, 25-28.

Harmon, S.A., Marnell, L.L. and Summers, D.F. (1983) The major ribonucleoprotein-associated protein kinase of vesicular stomatitis virus is a host cell protein. J. Biol. Chem. 258, 15283-15290.

Holbrook, H.J., Carlson, S.G., Choi, A.M.K. and Fargnoli, J. (1992) Induction of *HSP70* gene expression by the antiproliferative prostaglandin PGA_2: a growth-dependent response mediated by activation of heat shock transcription factor. Mol. Cell. Biol. 12, 1528–1534.

Imblum, R.G. and Wagner, R.R. (1974) protein kinase and phosphoprotein of vesicular stomatitis virus. J. Virol. 13, 113–124.

Jameson, P., Dixon, M. and Grossberg, S.E. (1977) A sensitive assay for many species of cells: encephalomyocarditis virus hemaggluatination yield reduction. Proc. Soc. Exp. Biol. Med. 155, 173–178.

Kalebic, T., Kinter, A., Poli, G., Anderson, M.E., Meister, A. and Fauci, A. (1991) Suppression of human immuno deficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester and N-acetylcysteine. Proc. Natl. Acad. Sci. USA 88, 986–990.

Koizumi, T., Negishi, M. and Ichikawa, A. (1992a) Induction of heme oxygenase by Δ^{12} prostaglandin J_2 in porcine aortic endothelial cells. Prostaglandins 43, 121–131.

Koizumi, T., Negishi, M. and Ichikawa, A. (1992b) Inhibitory effect of an intracellular glutathione on Δ^{12} prostaglandin J_2-induced protein syntheses in porcine aortic endothelial cells. Biochem. Pharm. 44, 1597–1602.

Langford, M.P., Weigent, D.A., Stanton, G.J. and Baron, S. (1981) Virus plaque-reduction assay for interferon: microplaque and regular macroplaque reduction assays. Methods Enzymology 78, 339–351.

Luczak, M., Bumulka, W., Szmigielski, S. and Korbecki, M. (1975) Inhibition of multiplication of parainfluenza 3 virus in prostaglandin-treated WISH cells. Arch. Virol. 49, 377–380.

Massey, D.M. and Lenard, J. (1987) Inactivation of the RNA polymerase of vesicular stomatitis virus by N-ethylmaleimide and protection by nucleoside triphosphates. J. Biol. Chem. 262, 8734–8737.

Mastromarino, P., Conti, C., Petruzziello, R., DeMarco, A., Pica, F. and Santoro, M.G. (1993) Inhibition of Sindbis virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat-shock protein synthesis. Antiviral Res. 20, 209–222.

Meister, A. (1988) Glutathione metabolism and its selective modification. J. Biol. Chem. 263, 17205–17208.

Ohno, K., Fukushima, M., Fujiwara, M. and Narumiya, S. (1988) Induction of 68000-dalton heat shock proteins by cyclopentenone prostaglandins. Its association with prostaglandin-induced G_1 block in cell cycle progression. J. Biol. Chem. 263, 19764–19770.

Ohno, K. and Hirata, M. (1990) Induction of γ-glutamulcysteine synthetase by prostaglandin A_2 in L-1210 cells. Biochem. Biophys. Res. Commun. 168, 551–557.

Parker, J. and Ankel, H. (1992) Formation of a prostaglandin A_2-glutathione conjugate in L1210 mouse leukemia cells. Biochem. Pharmacol. 43, 1053–1060.

Pica, F., DeMarco, A., DeCesare, F., and Santoro, M.G. (1993) Inhibition of vesicular stomatitis virus replication by Δ^{12} prostaglandin J_2 is regulated at two separate levels and in association with induction of stress protein synthesis. Antiviral Res. 20, 193–208.

Polet, H. and Levine, L. (1975) Metabolism of prostaglandins E, A, and C in serum. J. Biol. Chem. 250, 351–357.

Santoro, M.G., Jaffe, B.M. and Esteban, M. (1983) Prostaglandin A inhibits the replication of vesicular stomatitis virus: effect on virus glycoprotein. J. Gen. Virol. 64, 2797–2801.

Santoro, M.G., Garaci, E., and Amici, C. (1989) Prostaglandins with antiproliferative activity induce the synthesis of a heat shock protein in human cells. Proc. Natl. Acad. Sci. USA 86, 8407–8411.

Santoro, M.G., Garaci, E., and Amici, C. (1990) Induction of HSP70 by prostaglandins; In: M.J. Schlesinger, M.G. Santoro, E. Garaci, (Eds), Stress Proteins: Induction and Function. pp 27–34 Springer-Verlag, Berlin.

Stehle, R.G. and Oesterling, T.O. (1977) Stability of prostaglandin E_1 and dinoprostone (prostaglandins E_2) under strongly acidic and basic conditions. J. Pharm. Sci. 66, 1590–1595.

Takacs, A.M., Barik, S., Das, T. and Banerjee, A.K. (1992) Phosphorylation of specific serine residues within the acidic domain of the phosphoprotein of vesicular stomatitis virus regulates transcription in vitro. J. Virol. 66, 5842–5848.

Yamamoto, N., Fukushima, M., Tsurumi, T., Maeno, K. and Nishiyama, Y. (1987) Mechanism of inhibition of herpes simplex virus replication by Δ^7prostaglandin A_1 and Δ^{12}-prostaglandin J_2. Biochem. Biophys. Res. Commun. 146, 1425–1431.