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Inhibition of vesicular stomatitis virus replication by Δ^{12}-prostaglandin J_2 is regulated at two separate levels and is associated with induction of stress protein synthesis

F. Pica[a], A. De Marco[a], F. De Cesare[a] and M.G. Santoro[a,b]

[a]*Institute of Experimental Medicine, C.N.R., Viale C. Marx 15, 00156 Rome, Italy and* [b]*Department of Experimental Medicine, University of L'Aquila, Collemaggio, 67100 L'Aquila, Italy*

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Summary

Δ^{12}-Prostaglandin J_2 (Δ^{12}-PGJ_2), a naturally occurring dehydration product of prostaglandin D_2, is shown to suppress the replication of vesicular stomatitis virus (VSV) in two different epithelial monkey cell lines. A significant delay in the virus-induced cytopathic effect and a dramatic inhibition of virus production can be obtained at doses which do not inhibit protein synthesis in uninfected cells, and induce the synthesis of heat shock proteins (HSPs) in both uninfected and VSV-infected cells. Δ^{12}-PGJ_2 is shown to block VSV replication at two separate levels in the early and late phase of the virus replication cycle. Treatment started soon after VSV infection greatly suppresses viral (but not cellular) protein synthesis and prevents the virus-induced shut-off of host cell protein synthesis. This effect is accompanied by the induction of HSP synthesis. Δ^{12}-PGJ_2-treatment in a late phase of the virus replication cycle, when all virus proteins have been synthesized, still causes a dramatic block of infectious virus production. This block is accompanied by a decrease in [^{3}H]glucosamine incorporation into the virus glycoprotein G, at concentrations which do not alter glucosamine uptake by the cells, suggesting that a defect in virus protein glycosylation could be responsible for the antiviral activity. Finally, Δ^{12}-PGJ_2 causes a decrease of glucosamine incorporation into at least two host cell polypeptides, while the majority of cellular proteins are unaffected and glycosylation of a 47 kDa cellular protein is strongly induced. These

Correspondence to: M.G. Santoro, Institute of Experimental Medicine, II University of Rome, Via O. Raimondo, Rome, Italy. Fax: 0039-6-7234793

selective alterations of protein glycosylation suggest that Δ^{12}-PGJ_2 affects a specific group of glycosylated proteins. The finding that cyclopentenone prostaglandins act on different events during the virus cycle explains the effectiveness of these compounds in controlling the replication of different types of viruses and presents an attractive new approach to antiviral chemotherapy.

Cyclopentenone; Glycosylation; Prostaglandin; Stress protein; Vesicular stomatitis virus

Introduction

Prostaglandins (PGs), a class of naturally occurring cyclic 20-carbon fatty acids, which function as microenvironmental hormones and intracellular signal mediators, are known to participate in the regulation of a variety of physiological and pathological processes, among which are inflammation (Vane, 1987), the immune response (Ninnemann, 1988), fever production (Dinarello and Wolf, 1982) and virus replication (Santoro, 1987).

Prostaglandins containing an α,β-unsaturated carbonyl group in the cyclopentane ring structure (cyclopentenone PGs, i.e., PGAs and PGJs) have been shown to possess a potent antiviral activity against a wide variety of DNA and RNA viruses, including Poxviruses (Santoro et al., 1982a), Herpesviruses (Santoro, 1987; Yamamoto et al., 1987), Paramyxoviruses (Santoro et al., 1980; Amici and Santoro, 1991), Ortomyxoviruses (Santoro et al., 1988), Picornaviruses (Ankel et al., 1985), Rhabdoviruses (Santoro et al., 1983), Togaviruses (Mastromarino et al., 1992) and Retroviruses (D'Onofrio et al., 1990a,b; Ankel et al., 1991), in different types of mammalian cells. In the majority of the models studied, the target for the antiviral activity appeared to be a late event in the virus replication cycle. PGAs have been shown to cause alterations in the synthesis and/or maturation of one or more virus proteins in vaccinia, VSV and Sendai viruses (Santoro et al., 1982a, 1983, 1989a; Benavente et al., 1984). A PGA_1-mediated block in an early stage of virus infection has been shown in human embryo fibroblasts infected with Herpes virus type I (Yamamoto et al., 1987) and recently in L-1210 cells infected with vesicular stomatitis virus (Bader and Ankel, 1990), both describing a block of virus RNA transcription.

In the present report, we describe the antiviral activity of Δ^{12}-PGJ_2 (9-deoxy-Δ^9,Δ^{12}-13,14-dihydro-PGD_2), a natural metabolite of PGD_2 (Kikawa et al., 1984), which presently occurs in human body fluids (Hirata et al., 1988), using as a model the epithelial monkey cell line MA104, infected with vesicular stomatitis virus. The results describe how Δ^{12}-PGJ_2-treatment blocks virus replication at two separate steps, in the early and late phases of the replication cycle, respectively, while strongly inducing stress protein synthesis.

Materials and Methods

Cell cultures and virus infection

MA104 rhesus kidney cells obtained from the laboratory of Dr. Enrique Rodriguez-Boulan (Cornell University, Medical College, New York, NY) and 37RC African Green monkey kidney cells were grown in either T-25 Falcon flasks or in 24-well Linbro plates in RPMI 1640 culture medium (Gibco) supplemented with 10% fetal calf serum (FCS, Gibco) and antibiotics at 37°C in a 5% CO_2 atmosphere. Confluent MA104 and 37RC monolayers were washed with phosphate-buffered saline (PBS) and infected with VSV (3 PFU/cell), kindly provided by Dr. E. Rodriguez-Boulan. After incubation for 1 h at 37°C, virus inocula were removed and monolayers were washed three times with PBS and incubated with 1 ml of RPMI 1640 containing 2% FCS and prostaglandin or control diluent. Δ^{12}-PGJ_2 (Cayman Chemical) was stored as a 100% ethanolic stock solution (1 mg/ml) at −20°C and was diluted to the appropriate concentration immediately before use. Control media contained the same concentration of ethanol diluent, which was shown not to affect cell or virus metabolism.

Virus titration

For VSV titration, medium was collected 24 h post-infection (p.i.) and, after removing cell debris by centrifugation at 2000 rpm for 15 min, titers were determined by cytopathic effect 50% (CPE 50%) assay on confluent monolayers of MA104 or 37RC cells in 96-well tissue culture dishes as previously described in detail (Rodriguez-Boulan, 1983). The dilution that gives 50% cytopathic effect was determined by the interpolating procedure of Reed and Muench (Rodriguez-Boulan, 1983).

Protein labelling and SDS/PAGE

At different times after VSV infection, confluent cell monolayers were labelled with [^{35}S]methionine (10 μCi/2 × 10^5 cells, unless otherwise specified) in methionine-free medium containing 5% dyalised FCS. Cells were usually preincubated for 15–30 min in methionine-free medium. For glycoprotein synthesis cell monolayers were labelled with [^{3}H]glucosamine hydrochloride (20 μCi/2 × 10^5 cells, Amersham). After labelling, cells were washed, lysed in lysis buffer (2% SDS, 10% glycerol, 0.001% Bromophenol Blue, 0.1 M DTT, 0.0625 M Tris-HCl (pH 6.8)) and the radioactivity incorporated was determined as previously described (Santoro et al., 1989a). Samples were analysed by SDS/PAGE in a vertical slab gel apparatus (3% stacking gel, 7.5% resolving gel, unless otherwise specified) using the buffer system described by Laemmli (1970). Gels were washed, fixed in 10% acetic acid, 10% TCA and 30% methanol, dried under vacuum and autoradiographed using Kodak films (Eastman Kodak). The M_rs values of polypeptides were calculated by comparison with the following markers: myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (68 kDa),

ovalbumin (43 kDa), carbonic anhydrase (31 kDa) and lysozyme (14 kDa).

Immunoblot analysis

For immunoblot analysis, an equal amount of protein for each sample was separated by SDS/PAGE as described above, and blotted onto nitrocellulose using the technique described by Burnette (1981). After transfer, filters were incubated with an anti-72/73 kDa HSP monoclonal antibody (diluted 1:500) from HeLa cells (Amersham), which has been shown to be reactive against human and monkey HSP70, in TEN-Tween 20 buffer, (0.05 M Tris-HCl (pH 7.4), 5 mM EDTA, 0.15 M NaCl, 0.05% Tween 20) and the bound antibody was detected by horseradish peroxidase-linked sheep anti-mouse antibody. M_rs were calculated using Bio-Rad low M_r markers. For quantitative determination of protein synthesis, after immunodetection, the individual bands were excised from the blots and the radioactivity incorporated was measured in a β-scintillation counter, as previously described (Amici and Santoro, 1991).

Statistical analysis

Statistical analysis was performed using the Student's *t*-test for unpaired data. Data were expressed as the mean $\pm$ S.E. and P values of <0.05 were considered significant.

Results

Effect of Δ^{12}-PGJ$_2$ on VSV replication

Confluent monolayers of MA104 cells were infected with VSV (3 PFU/cell), and treated with different concentrations of Δ^{12}-PGJ$_2$, starting after the 1 h infection period. VSV cytopathic effect which causes cell rounding and detachment from substrata (Fig. 1c) started to be evident in all cells 8–10 h post-infection (p.i.) and was totalled after 24 h. In these conditions, virus replication cycle was completed by 8–10 h, at which time infectious VSV could be found in the supernatant of infected cells. Virus production was maximal at 24 h p.i.. Supernatants were collected 24 h p.i. and virus titers were determined by the CPE 50% assay. Fig. 1a shows that Δ^{12}-PGJ$_2$ inhibits virus replication dose-dependently with concentrations of 1–2 μg/ml being able to reduce virus yield by 50%, and doses between 8 and 10 μg/ml being effective in causing a 90–99% inhibition of virus production. The concentration of 8 μg/ml was found to be the most active nontoxic dose, not altering cell viability or protein synthesis in uninfected cells, and was used in all the following experiments. Δ^{12}-PGJ$_2$ at the concentration of 8 μg/ml, even though it did not prevent the appearance of the virus-induced CPE, it delayed it for several hours, and a majority of treated cells appeared to be still unaffected after 16 h p.i. (Fig. 1c). In order to determine whether Δ^{12}-PGJ$_2$ was acting on an early or on a late event during VSV replication cycle, MA104 cells were infected with VSV and treated with Δ^{12}-PGJ$_2$ (8 μg/ml) at different times p.i.. Fig. 1b shows that

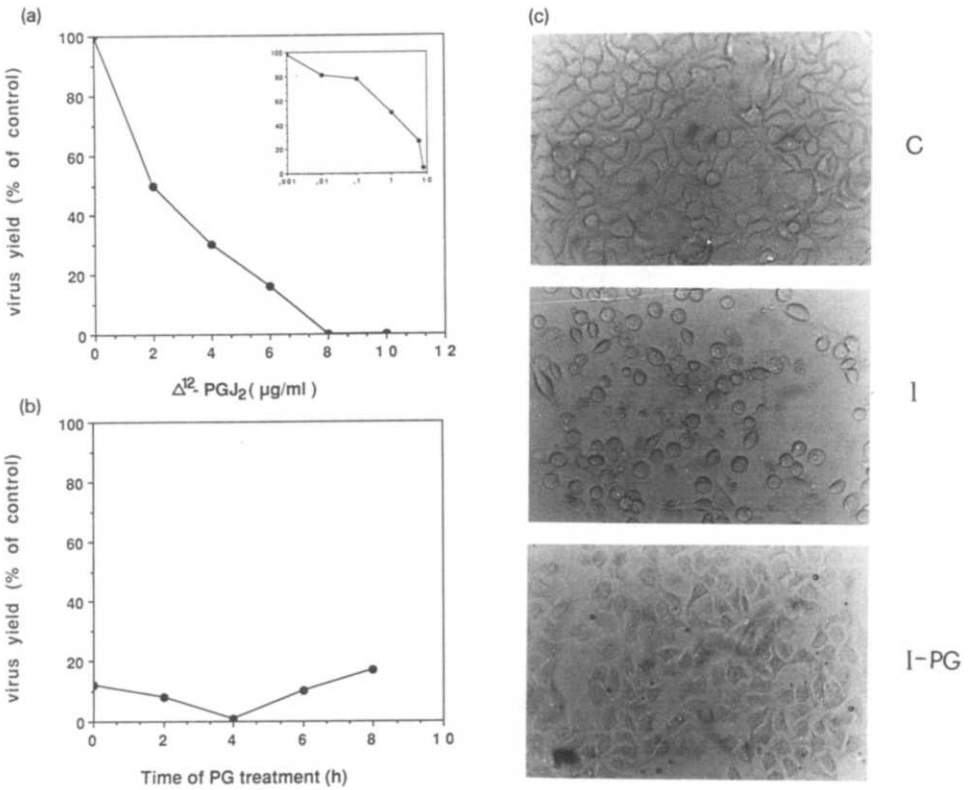

Fig. 1. Effect of Δ^{12}-PGJ_2 on VSV replication in MA104 cells. (a) Dose-dependent inhibition of VSV-production 24 h p.i.. The effect of doses lower than 2 μg/ml is shown in the insert. Virus yield of control was $3.35 \pm 0.22 \times 10^7$ CPE-50% units/ml. (b) Effect of Δ^{12}-PGJ_2-treatment (8 μg/ml) started at different times after VSV infection. (c) Effect of Δ^{12}-PGJ_2 on VSV-induced cytopathic effect 16 h p.i. C = uninfected control; I = VSV-infected; I-PG = VSV-infected, treated with Δ^{12}-PGJ_2 (8 μg/ml).

inhibition of VSV replication could be obtained even when treatment was started as late as 8 h p.i., indicating a rapid effect on a late stage of virus replication.

Effect of Δ^{12}-PGJ_2 on protein synthesis and HSPs induction

Previous reports have shown that the effect of antiviral prostaglandins (PGAs and PGJ_2) was related to alterations of the synthesis and/or maturation of specific virus proteins. In order to determine whether Δ^{12}-PGJ_2 could be acting by specifically affecting the synthesis of one or more VSV proteins, MA104 cells were infected with VSV and treated with Δ^{12}-PGJ_2 (8 μg/ml), PGA_1 (15 μg/ml) or control diluent soon after the 1 h infection period. Cells were labelled with [^{35}S]methionine (10 μCi/2×10^5 cells) starting 4 h p.i. for the following 4 h. After this time, cells were lysed in lysis buffer and the radioactivity incorporated into the TCA-insoluble material was determined.

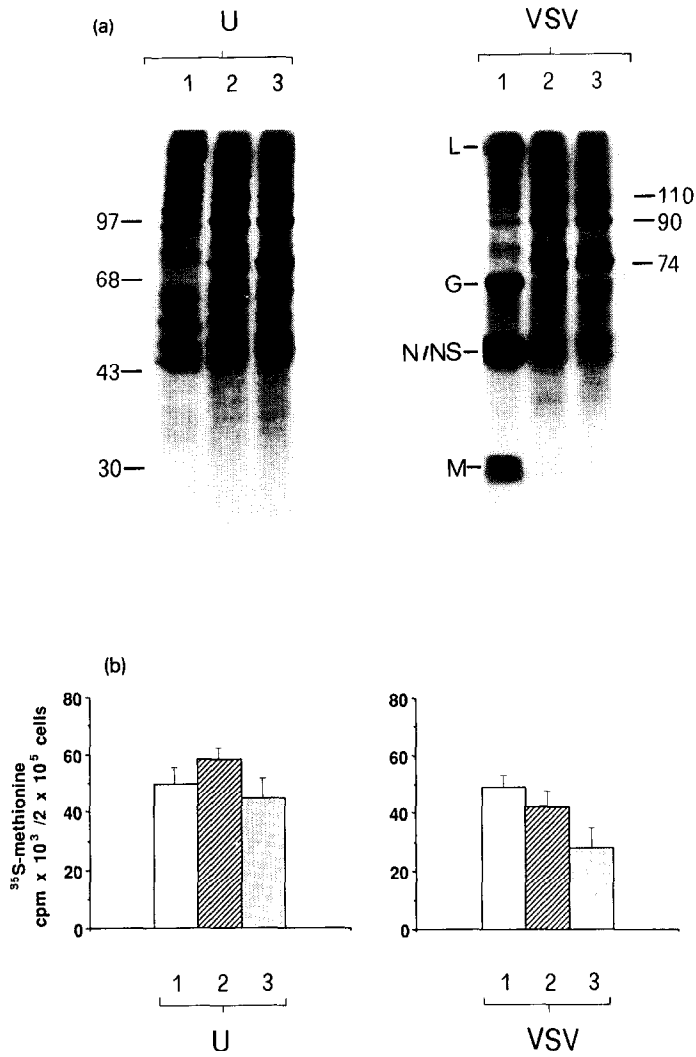

Fig. 2. Effect of early Δ^{12}-PGJ_2-treatment on the synthesis of cellular and viral proteins in MA104 cells infected with VSV. Uninfected (U) or VSV-infected (VSV) MA104 cells were treated with PGA_1 (2), Δ^{12}-PGJ_2 (3) or control diluent (1) soon after VSV infection and labelled with [^{35}S]methionine (4 h pulse starting 4 h p.i.). (a) SDS-PAGE analysis of radiolabeled polypeptides. Early treatment completely blocked VSV protein synthesis and induced the synthesis of three cellular polypeptides 110, 90 and 74 kDa M_r, respectively. (b) Incorporation of [^{35}S]methionine into TCA-insoluble material of samples described in (a). Data represent the mean $\pm$ S.E. of duplicate samples.

Samples containing the same amount of radioactivity were processed for SDS/PAGE and autoradiography. Fig. 2 shows that Δ^{12}-PGJ_2, as well as PGA_1 did not inhibit protein synthesis in uninfected MA104 cells (Fig. 2b). Treatment with cyclopentenone PGs, however, induced the synthesis of at least three new

polypeptides of molecular weight 74 kDa, 90 kDa and 110 kDa, respectively, (Fig. 2a). In VSV-infected cells, Δ^{12}-PGJ_2 caused a partial inhibition of protein synthesis (Fig. 2b). SDS/PAGE analysis surprisingly revealed that treatment with both PGA_1 and Δ^{12}-PGJ_2 dramatically suppressed the synthesis of virus proteins, while it prevented the virus-induced shut-off of cellular protein synthesis (Fig. 2a). Both cyclopentenone PGs markedly induced the synthesis of a 74 kDa, 90 kDa and 110 kDa cellular polypeptides in VSV-infected as well as in uninfected MA104 cells (Fig. 2a).

We have previously shown that PGA_1 and PGJ_2 are able to induce the synthesis of a 74 kDa protein in both monkey 37RC cells (Santoro et al., 1982b; Santoro et al., 1987) and human erythroleukemic K562 cells (Santoro et al., 1986). This protein has been identified as a heat shock protein (HSP) belonging to the 70 kDa HSP family (HSP70) (Santoro et al., 1989b; Amici and Santoro, 1991). In order to verify whether the 74 kDa protein, the synthesis of which was induced by Δ^{12}-PGJ_2 in MA104 cells, could be a HSP70, MA104 cells uninfected or infected with VSV were treated with Δ^{12}-PGJ_2 (8 μg/ml) starting soon after infection. After 24 h proteins were extracted and an equal amount of protein from different samples was separated by SDS/PAGE and processed for immunoblot analysis using anti-human 72/73 kDa HSP monoclonal antibodies. Immunoblot analysis revealed the presence of a unique band of approx. 74 kDa molecular weight in uninfected and VSV-infected cells treated with Δ^{12}-PGJ_2 (Fig. 3), identifying the 74 kDa protein as an HSP70. The 90 kDa and 110 kDa proteins induced by prostaglandins have also been identified as heat shock proteins by comparison with HSPs induced by a 20 min heat shock at 45°C, in uninfected as well as VSV-infected MA104 cells (De Marco and Santoro, submitted).

Kinetics of VSV protein synthesis is shown in Fig. 4. MA104 cells were

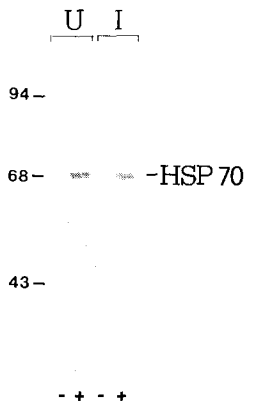

Fig. 3. Induction of HSP70 by Δ^{12}-PGJ_2. MA104 uninfected (U) or infected with VSV were treated with 8 μg/ml Δ^{12}-PGJ_2 (+) or control diluent (-) starting soon after infection. After 24 h, proteins were extracted and an equal amount of protein for each sample was separated by SDS/PAGE and processed for immunoblot analysis using anti-human 72/73 kDa HSP monoclonal antibodies.

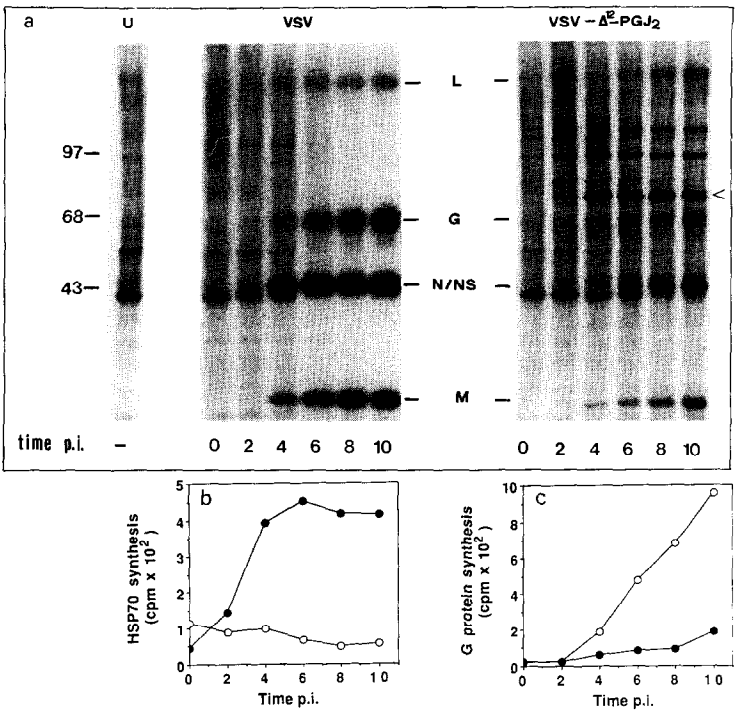

Fig. 4. Kinetics of VSV protein synthesis. MA104 cells infected with VSV (3 PFU/cell), were treated with Δ^{12}-PGJ_2 (8 μg/ml) or control diluent after the 1 h adsorption period and labelled with [^{35}S]methionine (10 μCi/2 $\times$ 10^5 cells, 1 h pulses) at different intervals p.i. (a) SDS-PAGE analysis and autoradiography of samples containing the same amount of radioactivity. U = uninfected cells; VSV = infected, untreated cells labelled at 0, 2, 4, 6, 8 and 10 h p.i.; VSV-Δ^{12}-PGJ_2 = VSV-infected, Δ^{12}-PGJ_2-treated cells labelled at 0, 2, 4, 6, 8 and 10 h p.i.. HSP70 is indicated by arrow. HSP70 synthesis (b) and VSV G glycoprotein synthesis (c) were quantified in control (○) or Δ^{12}-PGJ_2-treated (●) VSV-infected MA104 cells, by cutting out the relevant bands from dried gels and counting the radioactivity in a β-scintillation counter.

infected with VSV and treated with Δ^{12}-PGJ_2 (8 μg/ml) or diluent control soon after infection. Cells were labelled with [^{35}S]methionine (10 μCi/2 $\times$ 10^5 cells, 1 h pulses) at different intervals after infection. After determining the radioactivity incorporated into the TCA-insoluble material, samples containing the same amount of radioactivity were separated on SDS/PAGE and processed for autoradiography. Virus protein synthesis was maximal between 6 and 10 h p.i., and by 6 h p.i. the shut-off of cellular protein synthesis was completed (Fig. 4a). Δ^{12}-PGJ_2 treatment dramatically inhibited VSV protein synthesis at all times p.i. and prevented the shut-off of cellular protein synthesis up to 10 h p.i. (Fig. 4a,c). Δ^{12}-PGJ_2-induced HSP70 synthesis was found to start at 2 h p.i. and to continue up to 10 h p.i. (Fig. 4a, b).

Effect of Δ^{12}-PGJ_2 on virus protein glycosylation

The results described above demonstrated that Δ^{12}-PGJ_2-treatment can

result in the block of virus protein synthesis; they, however, did not explain the effect of Δ^{12}-PGJ_2 treatment started as late as 6 or 8 h p.i., when virus proteins have already been synthesized. As shown in Fig. 1, Δ^{12}-PGJ_2 treatment in late phases of virus replication still resulted in the block of infectious virus production. This suggested that a second late event in the virus replication cycle, independent from virus protein synthesis, could also be affected by Δ^{12}-PGJ_2. Since it has been previously shown that PGA_1 affects the glycosylation of HN and F_0 glycoproteins of Sendai virus (Santoro et al., 1989a), the possibility that the glycosylation of the G protein of VSV could be affected was investigated.

MA104 cells infected with VSV were treated with Δ^{12}-PGJ_2 at different times after infection and labelled with either [^{35}S]methionine (10 μCi/2 $\times$ 10^5 cells, 2 h pulse starting 8 h p.i.) or [^{3}H]glucosamine (20 μCi/2 $\times$ 10^5 cells, 4 h pulse starting 6 h p.i.). After labelling, cells were washed three times in PBS and lysed in lysis buffer. After determination of the radioactivity incorporated into TCA-insoluble material, samples containing the same amount of radioactivity were separated on SDS/PAGE and processed for autoradiography. Fig. 5a shows

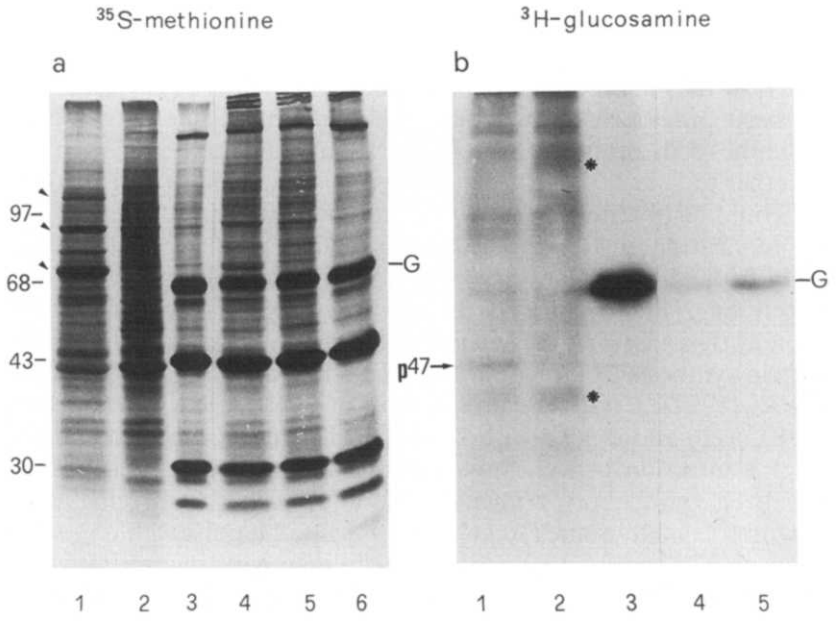

Fig. 5. Effect of Δ^{12}-PGJ_2 treatment in the late stages of VSV infection on virus protein synthesis and glycosylation. Uninfected (lanes 1, 2) or VSV-infected (lanes 3–6) MA104 cells were treated with Δ^{12}-PGJ_2 (lanes 1, 4, 5 and 6) or control diluent (lanes 2 and 3) at different times p.i., and labelled with [^{35}S]methionine (2 h pulse starting at 8 h p.i.), or [^{3}H]glucosamine (4 h pulse starting at 6 h p.i.). Treatment with Δ^{12}-PGJ_2 was started at 4, 6 or 8 h p.i. (lanes 4, 5 or 6, respectively). PG-treatment started at 6 or 8 h p.i. had little effect on VSV protein synthesis, but caused a dramatic block of VSV G protein glycosylation. Δ^{12}-PGJ_2 also inhibited the glycosylation of two cellular polypeptides (indicated by asterisks), while induced the glycosylation of a 47 kDa cellular protein (indicated by arrow).

that Δ^{12}-PGJ_2 treatment started at 4 or 6 h p.i. still partially protected host protein synthesis by the virus-induced shut-off. This effect was not obtained if treatment was started 8 h p.i.. Moreover, Δ^{12}-PGJ_2 treatment started after 4 h p.i. did not affect virus protein synthesis as measured by [^{35}S]methionine incorporation (Fig. 5a), but it dramatically inhibited the glycosylation of the G protein as measured by [^{3}H]glucosamine incorporation (Fig. 5b). This effect was obtained without producing an overall inhibition of cellular protein glycosylation in uninfected cells ([^{3}H]glucosamine incorporation into proteins: Control = 4.76 ± 0.72; PG-treated = 4.53 ± 0.35 cpm $\times 10^4/2 \times 10^5$ cells), as well as in VSV-infected cells (Control = 2.11 ± 0.05; PG-treatment started at 4 h = 2.07 ± 0.01; PG-treatment started at 6 h = 1.92 cpm $\times 10^4/2 \times 10^5$ cells). Interestingly, Δ^{12}-PGJ_2 also specifically inhibited the glycosylation of at least two cellular polypeptides (100 kDa and 40 kDa M_r), while it caused the glycosylation of a 47 kDa cellular protein (p47) (Fig. 5c). Alteration of protein glycosylation was not due to inhibition of [^{3}H]glucosamine uptake by MA104 cells either uninfected (Control = 5.91 ± 0.03; PG-treated = 6.23 ± 0.05 c.p.m. $\times 10^4/2 \times 10^5$ cells) or infected with VSV (Control = 6.27 ± 0.61; PG-treated = 6.06 ± 0.48 c.p.m. $\times 10^4/2 \times 10^5$ cells). These data suggest an effect on specific steps of the glycosylation process, which are relatively uncommon in cellular glycoproteins.

Effect of Δ^{12}-PGJ_2 on VSV replication in monkey kidney 37RC cells

It has been previously shown that PGA_1-treatment does not inhibit VSV protein synthesis in mouse L fibroblasts (Santoro et al., 1983). In order to verify whether the block of VSV protein synthesis was characteristic of MA104 cells or it could be obtained in other cell lines, we studied the effect of Δ^{12}-PGJ_2 on VSV replication in a monkey kidney cell line, 37RC, which has been extensively studied in our laboratory as a model for Paramyxovirus infection. In this cell line, PGA_1 as well as Δ^{12}-PGJ_2 could suppress Sendai virus replication at doses lower than the one used in MA104 cells without inhibiting virus protein synthesis 24 h p.i. (Santoro et al. 1989a; Amici and Santoro, 1991; Amici et al., 1992). Confluent monolayers of 37RC cells were infected with VSV (3 PFU/cell) and treated with Δ^{12}-PGJ_2 (4 μg/ml), or diluent control, soon after the 1 h infection period. Supernatants were collected 24 h p.i. for virus titration. In a parallel experiment, VSV-infected or uninfected cells were labelled with [^{35}S]methionine (10 μCi/2×10^5 cells, 2 h pulse) 8 h p.i.. After a 4 h incubation at 37°C, cells were lysed, the radioactivity incorporated into the TCA-insoluble material was determined, and proteins were processed for SDS/PAGE and autoradiography. Fig. 6 shows that in these cells, a lower dose of Δ^{12}-PGJ_2 (4 μg/ml) was needed to obtain a dramatic suppression of VSV production (Fig. 6a). Δ^{12}-PGJ_2 at this dose did not inhibit protein synthesis in either VSV-infected or uninfected 37RC cells (Fig. 6b), but specifically blocked VSV protein synthesis and protected the host cell by the virus induced shut-off of protein synthesis (Fig. 6c). Moreover, Δ^{12}-PGJ_2 also induced in both uninfected and VSV-infected 37RC cells the synthesis of a 110 kDa, 90 kDa

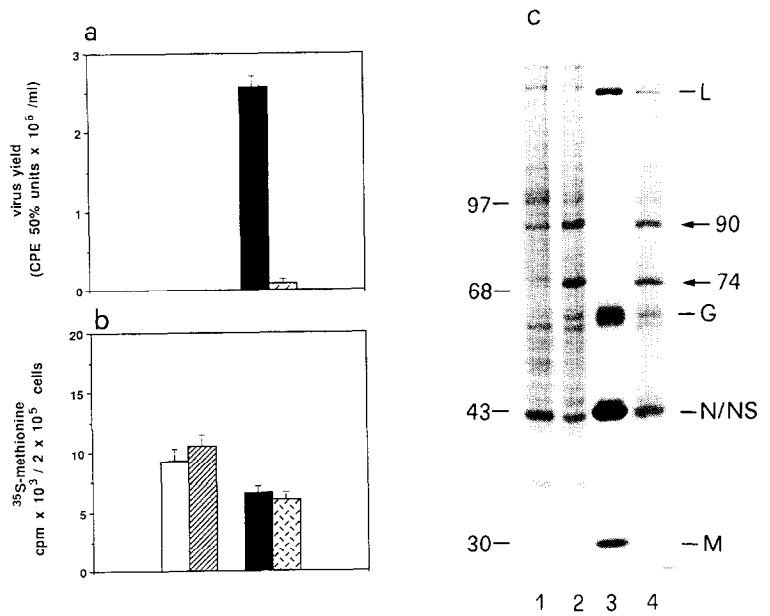

Fig. 6. Effect of Δ^{12}-PGJ_2 treatment on VSV-replication in 37RC cells. 37RC cells uninfected or infected with VSV were treated with Δ^{12}-PGJ_2 (4 μg/ml) or diluent control after the 1 h adsorption period. (a) Virus yield in the supernatant of untreated (■) or Δ^{12}-PGJ_2-treated (▨) cells 24 h p.i.. Cells were labelled with [^{35}S]methionine (10 μCi/2 × 10^5 cells, 2 h pulse) 8 h p.i.. (b) [^{35}S]methionine incorporation into TCA-insoluble material of uninfected untreated cells (1, □), uninfected cells treated with Δ^{12}-PGJ_2 (2, ▨), VSV-infected cells (3, ■) and VSV-infected PG-treated cells (4, ▨). (c) SDS-PAGE analysis and autoradiography of samples described in (b).

and 74 kDa cellular proteins, identified as stress proteins by immunoblot analysis (data not shown).

Discussion

In 1982 Fukushima et al. described a 9-deoxy-Δ^9 derivative of PGD_2, characterized by a potent antineoplastic and a weak smooth muscle-contracting activity, which was obtained by incubation of PGD_2 in aqueous solution for 24 h. In these conditions, PGD_2 underwent dehydration, and the product formed, which, as in the case of conversion of PGE_2 to PGA_2, contained a cyclopentenone ring, was named PGJ_2 (Fukushima et al., 1982). The presence of human plasma during incubation of PGD_2 unexpectedly caused the formation of a new compound, a 9-deoxy-Δ^9, Δ^{12}-13,14-dihydro-PGD_2, in which the double bond of PGJ_2 at $C_{13,14}$ was shifted to $C_{12,13}$, and which was consequently designated as Δ^{12}-PGJ_2 (Kikawa et al., 1984; Fitzpatrick and Wynalda, 1983). Δ^{12}-PGJ_2 was characterized by potent antineoplastic activity, as PGJ_2, but had no smooth muscle-contracting activity or inhibitory activity

on platelet aggregation (Kikawa et al., 1984; Narumiya and Toda, 1985). Δ^{12}-PGJ_2 has been shown to be physiologically present in humans and normal levels have been quantitated in human body fluids (Hirata et al., 1988). Recently, an antiviral activity of Δ^{12}-PGJ_2, at concentrations which did not cause strong pharmacological side effects, has been found in a mouse model after infection with PR8 influenza A virus (Pica et al., in preparation).

Analysis of uptake and intracellular distribution has shown that Δ^{12}-PGJ_2 is rapidly incorporated into cells, reaching an intracellular concentration 20 times higher than in the incubation medium, suggesting a carrier mechanism similar to the one used for uptake of glucose, thymidine or leucine, and is transported into the nucleous at 37°C (Narumiya and Fukushima, 1986; Narumiya et al., 1986). It has also been suggested that Δ^{12}-PGJ_2 incorporation into cells could be a glutathione (GSH) coupling reaction, since it is dependent on the level of intracellular GSH (Onho et al., 1989).

An antiviral activity of Δ^{12}-PGJ_2 has been shown in human embryo fibroblasts infected with herpes simplex virus type I (Yamamoto et al., 1987). In this model, Δ^{12}-PGJ_2 was shown to inhibit viral RNA transcription. We have now shown that Δ^{12}-PGJ_2 can block the replication of a RNA virus belonging to the Rhabdovirus group, VSV, at two separate levels, related to two distinct events in the virus replication cycle.

A treatment with Δ^{12}-PGJ_2 at a concentration which does not suppress protein synthesis in uninfected cells, if started in an early stage of infection, resulted in a block of VSV protein synthesis and in a partial protection of the host cell from the virus-induced shut-off of cellular protein synthesis. Δ^{12}-PGJ_2 treatment, even though delayed the virus-mediated CPE, did not prevent killing of infected cells, but dramatically reduced the production of infectious virus particles, thus preventing spreading of infection.

Δ^{12}-PGJ_2 at the same concentration also induced the synthesis of stress proteins. In particular, a 74 kDa polypeptide induced by Δ^{12}-PGJ_2 was identified by immunoblot analysis as a heat shock protein belonging to the HSP70 group. Synthesis of HSP70 appeared between 2 and 4 h after Δ^{12}-PGJ_2 treatment and was still actively synthesized after 10 h.

Stress proteins are a group of polypeptides the synthesis of which is induced in prokaryotic cells as well as in eukaryotic cells by heat shock (heat shock proteins) or other environmental stresses; they can be divided into five families with M_r of 15–30 kDa (low M_r HSP), 60 kDa (HSP60), 70 kDa (HSP70), 90 kDa (HSP90) and 100–110 kDa (HSP110), respectively. In the eukaryotic cell, HSPs are generally present as multigene families, consisting of closely related protein isoforms, with members being expressed in unstressed cells (constitutive HSPs) as well as following heat treatment (inducible HSPs) (Lindquist and Craig, 1988; Schlesinger, 1990). The structure of HSPs, and HSP70 in particular, has been highly conserved throughout evolution from bacteria to man (Lindquist and Craig, 1988). In eukaryotic cells, constitutive HSP70 proteins are needed for protein folding, assembly and intracellular transport, and can bind to and dissociate protein complexes in the presence of ATP.

Inducible HSP70s bind to partially denaturated proteins and protect cells from the effect of stress (Schlesinger, 1990). It has been recently shown that constitutive HSP70 binds reversibly to nascent polypeptide chains before they are properly folded and it is needed to chaperone unfolded proteins to and from specific cellular organelles, and it has been suggested that large amounts of inducible HSP70 could irreversibly bind to newly synthesized proteins, interfering with the translation process (Beckmann et al., 1990). Schlesinger et al. (1991) have also recently shown that the presence of HSP70 proteins during in vitro translation of the mRNA encoding the Sindbis virus capsid protein, interfered with normal polypeptide synthesis. It could then be argued that the large amount of Δ^{12}-PGJ_2-induced HSP70 could interfere with translation of VSV mRNAs. However, cellular protein synthesis does not appear to be significantly affected in this model by the presence of large amounts of HSP70, making this possibility unlikely. Since it has been recently shown that PGA_1 blocks VSV RNA transcription in L-1210 cells (Bader and Ankel, 1990), it is possible that transcription and not translation of viral mRNA is inhibited by Δ^{12}-PGJ_2.

The results described in this paper also show that Δ^{12}-PGJ_2 is able to block VSV replication by influencing a second step in the virus replication cycle, independently of the above discussed block of virus protein synthesis. Δ^{12}-PGJ_2 treatment in a late stage of infection, in fact, dramatically decreased the level of glucosamine incorporation into the synthesized virus G glycoprotein. This effect appeared to be rather specific for the G-protein and only two cellular polypeptides appeared to be affected, while a 47 kDa cellular polypeptide became glycosylated in the presence of Δ^{12}-PGJ_2. A 47 kDa glycosylated collagen-binding stress protein (HSP47), which is synthesized in chick and mammalian cells in response to heat shock, has been described by Nagata et al. (1986, 1988). The possibility that the Δ^{12}-PGJ_2-induced p47 could be identified as HSP47 is currently under investigation.

The mechanism by which Δ^{12}-PGJ_2 affects protein glycosylation is not known. Δ^{12}-PGJ_2 has been recently shown to inhibit [^{3}H]glucosamine incorporation also in HN and F_0 glycoproteins of Sendai virus in the african green monkey kidney cell line 37RC. Also in this case [^{3}H]glucosamine incorporation appeared to be selectively inhibited in a 118 kDa cellular glycoprotein and to be increased in a 47 kDa cellular glycoprotein, indicating a specific effect (Amici et al. 1992). In MA104 cells infected with a temperature sensitive mutant of VSV, prostaglandin A_1 treatment has also been recently shown to prevent G-protein transport to the cell membrane (Alicéa et al., manuscript in preparation). Both PGA_1 and Δ^{12}-PGJ_2 possess an α,β-unsaturated double bond adjacent to a keto group in the cyclopentane ring, which renders this portion of the molecule able to form Michael's adducts with cellular nucleophilics (reviewed in Fukushima, 1990) and to covalently bind to cysteine residues of proteins via a thioester bond (Ham et al., 1975; Khan and Sorof, 1990; Fukushima, 1990). VSV G protein normally binds 1–2 moles of fatty acid, predominantly palmitic acid, per moles of protein (Schmidt and

Schlesinger, 1979), through a thioester bond to cysteine (Sefton and Buss, 1987; Bonetti et al., 1989). We propose that Δ^{12}-PGJ_2 could bind to the G protein in the place of the natural fatty acid during protein maturation, and that this binding could provoke an alteration in the tertiary structure of the protein, preventing its maturation.

While it is well established that cyclopentenone prostaglandins are potent inhibitors of virus replication, the literature has described different effects on virus metabolism in different virus-host cell models. Some reports have described a block of virus RNA transcription (Yamamoto et al., 1987; Bader and Ankel, 1990), while others have demonstrated alterations in the synthesis and maturation of specific virus proteins (reviewed in Santoro et al., 1990). The finding that cyclopentenone prostaglandins can act on at least two separate events of the virus replication cycle, explains the discrepancies reported in the literature, and is a step further in the understanding of the mechanism of action of these natural antiviral compounds.

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