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Courte note

EFFECT OF PROSTAGLANDINS PGE2 AND PGFα2 ON THE MEAN PLAQUE SIZE OF BOVINE HERPESVIRUS 1

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Résumé

EFFET DES PROSTAGLANDINES PGE2 ET PGFα2 SUR LA TAILLE MOYENNE DES PLAGES PRODUITES PAR LE BOVINE HERPESVIRUS 1. – L'effet des prostaglandines PGE2 et PGFα2 sur le virus de la rhinotrachéite infectieuse bovine (bovine herpesvirus 1 ; BHV-1) a été étudié par la mesure de la taille moyenne des plages produites en culture de cellules par la souche IBR/Cu5. Trois concentrations des prostaglandines PGE2 et PGFα2 ont été utilisées : 0,1, 1 et 10 μg/ml. L'addition de chaque prostaglandine au milieu de culture provoque une augmentation de la taille des plages par rapport aux témoins, aux concentrations de 1 et 10 μg/ml pour la PGE2 et de 10 μg/ml pour la PGFα2.

Several stimuli of reactivation of bovine herpesvirus 1 (BHV-1) have already been identified. Parturition, transport and injection of dexamethasone are associated with an increase of corticosteroid blood levels in cattle. These support the hypothesis that glucocorticoids act as inducers of reactivation of BHV-1 (Sheffy and Davies 1972, Thiry *et al* 1985 c, 1987). Other stimuli of reactivation, ie superinfection with parainfluenza 3 (PI-3) (Mensik *et al* 1976) and infestation with *Dictyocaulus viviparus* (Msolla *et al* 1983, Thiry *et al* 1985 b), do not directly produce an increase of corticosteroid blood levels. They may represent stressful conditions for cattle, but other mechanisms may be proposed in these two cases. The possible implication of prostaglandins in the appearance of recurrent lesions was proposed by Hill (« skin trigger hypothesis », for a review see Hill 1985) for herpes simplex virus (HSV) infection of mice. This hypothesis suggests that lesions in peripheral tissues provoke the release of prostaglandins in these inflamed tissues. This release could promote the development of recurrent disease either by enhancing the spread of HSV in infected tissues (Harbour *et al* 1978, 1983) or by a suppressive effect on antibody dependent cell-mediated cytotoxicity against HSV-infected cells (Troffater and Daniels 1980). Without such a stimulus (skin trigger), the virus can be reactivated, but no clinical lesion is produced and the virus is eliminated by immune mechanisms. As PI-3 infection and *D viviparus* infestation provoke an inflammation of the respiratory mucosa, prostaglandins must be released and could be involved in the steps following the reactivation process. A release of prostaglandins is not sufficient to provoke reactivation of HSV in mice, but the stimuli of reactivation are different depending on the herpesvirus. It cannot be excluded that prostaglandins act as true stimuli of reactivation of other herpesviruses. The release of prostaglandins may be implicated in part to explain the reactivation-reexcretion process in these two conditions : superinfection with PI3 and infestation with *D viviparuş*. We studied the *in vitro* effect to two prostaglandins, PGE2 and PGFα2 on cell cultures infected with BHV-1. Their effects were estimated by the measurement of the mean plaque size of BHV-1. *In vitro* experiments are needed to study the mechanisms involved in reactivation. Unfortunately, as pointed out in a previous paper (Curvers *et al* 1985), no *in vitro* model of BHV-1 latency is available. The effect of prostaglandins was therefore studied in acutely infected cells.

IBR/Cu5 strain of BHV-1 was propagated in Georgia Bovine Kidney cells (GBK) as previously described (Thiry *et al* 1985 a). The virus was plaque-purified three times and passed twice before the experiment. Cells were grown in six well multidishes (Nunc-Gibco, Paisley, Great-Britain). Two experiments were carried out : one with PGE2 and the other with PGFα2. Prostaglandins (Sigma, St-Louis, USA) used were of the following concentrations :

Table 1 – Effect of prostaglandins PGE2 and PGFα2 on the mean plaque size of bovine herpesvirus 1.

	Prostaglandin concentrations (µg/ml)			
PGE2	0	0.1	1	10
N	34	50	52	52
mean (mm^2)	1.34	1.30	2.27	1.90
SD	0.50	0.37	0.51	0.51
Duncan's test	A[a]	A	B	C
PGFα2	0	0.1	1	10
N	56	51	51	51
mean (mm^2)	1.82	1.91	1.87	2.38
SD	0.60	0.59	0.75	0.59
Duncan's test	A[a]	A	A	B

a : Means with the same letter are not significantly different.

0.1, 1 and 10 µg/ml. Confluent monolayers were treated 24 hours before infection with the three concentrations of prostaglandins PGE2 and PGFα2. Untreated monolayers were kept as controls. GBK monolayers were infected with three tenfold dilutions of IBR/Cu5 strain (10^5 plaque forming units/ml). After one hour of incubation, the inoculum was removed and infected monolayers were given as nutrient minimum essential medium supplemented with 5 % bovine anti-BHV-1 serum. Each inoculum received one of the three concentrations of prostaglandins, PGE2 and PGFα2. Uninfected cells treated with the three concentrations of prostaglandins PGE2 and PGFα2 were kept as controls. Cells were fixed and stained after six days. Monolayers showing the optimal number of plaques were selected for measurement of the mean plaque size. Plaque sizes were measured as previously described (Curvers *et al* 1985), but a special computer device (described below) was used. Plaques were observed with an inverted microscope. Their circumference was drawn on an electromagnetic digitizer (Micro digi-pad, GTCO) connected via a RS-232 interface to an IBM XT computer. Each plaque area was computed by the program « Surface », developed by ourselves. Each plaque was measured three times and the arithmetic means were obtained. Approximately 50 plaques were measured for each treatment. One way analysis of variance and Duncan's multiple range test (Steel and Torrie 1960) were used as statistical tests.

The presence of each prostaglandin in the cell culture medium significantly influenced the plaque size of BHV-1 (one way analysis of variance : $P < 0.001$). Duncan's test allowed the distinction to be made between three concentrations of PGE2 or two concentrations of PGFα2 ($P < 0.05$) (table 1). PGE2 produced a significant increase in the mean plaque size at 1 and 10 µg/ml. At 1 µg/ml, the mean plaque size was significantly larger than at 10 µg/ml. Plaques produced in the presence of PGFα2 were significantly larger than the control plaques only at 10 µg/ml. No cytotoxic effect was observed on confluent GBK cell monolayer by routine examination with an inverted microscope at the various concentrations of each prostaglandin.

The two prostaglandins, PGE2 and PGFα2, are able to increase the mean plaque size of BHV-1. A difference was observed in action of each prostaglandin. PGE2 significantly increased the plaque size at 1 and 10 µg/ml, but PGFα2 only at 10 µg/ml. The same kind of difference was described for HSV (Harbour *et al* 1978). An increase of the mean plaque size may be due to two effects which may be additive, either an enhancement of virus replication or a stimulation of the spread of virus from cell to cell. We have not yet investigated the effect of prostaglandins on BHV-1 replication, but it has been demonstrated that prostaglandins enhance HSV spread in Vero cells (Harbour *et al* 1978). Glucocorticoid treatment of cattle latently infected by BHV-1 provokes virus reactivation very efficiently (Thiry *et al* 1985 a). However, a previous study has shown that dexamethasone decreases the mean plaque size of BHV-1 (Curvers *et al* 1985). Therefore, a positive effect on the mean plaque size is not a prerequisite for the identification of a reactivation stimulus. A positive effect *in vitro* on the replication and/or the propagation of BHV-1 and an effective *in vivo* role in HSV reactivation suggest that prostaglandins are involved in the BHV-1 reactivation process, either for its induction or for its amplification when respiratory mucosae are inflamed. This supports the hypothesis that other mechanisms not associated with an increase in glucocorticoid blood levels could provoke BHV-1 reactivation. Further *in vivo* experiments are now needed to confirm this hypothesis.

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Abstract

The effect of prostaglandins PGE2 and PGFα2 on infectious bovine rhinotracheitis (IBR) virus (bovine herpesvirus 1 ; BHV-1) was studied by using the measurement of the mean size of plaques produced in cell culture by IBR/Cu5 strain. Three concentrations (0.1, 1 and 10 μg/ml) of prostaglandins, PGE2 and PGFα2 were used. Each prostaglandin provoked and increase of the mean plaque size when compared to control plaques, at the concentrations 1 and 10 μg/ml for PGE2 and 10 μg/ml for PGFα2.

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