Inhibition by Indomethacin of in vitro Reactivation of Latent Herpes Simplex Virus Type 1 in Murine Trigeminal Ganglia

By ICHIRO KURANE,† YOSHINORI TSUCHIYA, TSUYOSHI SEKIZAWA† AND KATSUO KUMAGAI*

Department of Microbiology, Tohoku University School of Dentistry, and †Department of Neurology, Institute of Brain Disease, Tohoku University School of Medicine, Sendai, Japan

(Accepted 11 June 1984)

SUMMARY

Infectious virus could no longer be detected in the trigeminal ganglia removed from mice 25 days after infection with herpes simplex virus type 1 (HSV-1) by the lip route. When the ganglia were cultured in vitro for 1 or 2 days, infectious HSV-1 was again detected in the ganglia, indicating the reactivation of latent HSV-1. The effect of indomethacin on this reactivation was examined. When the ganglia were cultivated in the presence of $5 \times 10^{-4} \text{M}$ indomethacin, the appearance of infectious virus in the ganglia was almost completely inhibited. After removal of indomethacin, infectious virus appeared and the virus titre reached levels found in the ganglia cultured in indomethacin-free medium, indicating that the inhibition of the reactivation may not be due to the cytotoxic effect of indomethacin on the ganglionic cells. The inhibitory effect of indomethacin was only seen when it was added shortly after explantation. These results suggest that indomethacin affects some early processes of viral reactivation in the explanted ganglia. The synthesis of prostaglandins (PGE and PGB) found in the explanted ganglia was strongly suppressed by indomethacin, as was the viral reactivation. Other inhibitors of prostaglandin synthesis, i.e. tetracaine, mepacrine and mefenamic acid, also inhibited viral reactivation in the explanted ganglia. These results suggest that the inhibitory effect of indomethacin on the reactivation of latent HSV-1 may be due to the inhibition of prostaglandin synthesis, although it is possible that other cellular changes which could be caused by indomethacin contributed to the suppression of reactivation.

INTRODUCTION

As a consequence of initial infection, herpes simplex virus (HSV) has been shown to persist in a latent state in the sensory ganglia of animals (Stevens & Cook, 1971; Scriba, 1975; Green et al., 1981b) and humans (Baringer & Swoveland, 1973; Galloway et al., 1979). The host's immune response plays a critical role in controlling the acute phase of the ganglionic infection (Price et al., 1975; Openshaw et al., 1979). The latent state of HSV, however, can be maintained in the absence of neutralizing antibody (Sekizawa et al., 1980) and the role of immunity in the latent phase of ganglionic infection is still poorly understood although immunosuppressive agents can induce virus reactivation (Openshaw et al., 1979; Sekizawa & Openshaw, 1984). Latent HSV can also be reactivated by various stimuli such as local trauma (Hill et al., 1978), chemical stimuli (Harbour et al., 1983b), fever (Warren et al., 1940), u.v. light (Blyth et al., 1976; Openshaw et al., 1979) and stress (Merigan, 1974; Lehner et al., 1975), which probably produce no profound effect on either the systemic lymphoid systems or immune responses (Hill & Blyth, 1976). The mechanisms underlying reactivation of HSV by these stimuli have not been thoroughly investigated. A theory most often proposed to explain reactivation consists of three
sequential events: (i) a stimulus acts on latent infection in the ganglion to switch on the reactivation from the latent state; (ii) virus travels down the peripheral nerve; (iii) epidermal cells are infected and a skin lesion develops (ganglion trigger theory) (Cook & Stevens, 1973; Openshaw et al., 1982). Another theory has been proposed by Hill and Blyth. In this, they presume that infectious HSV is often released from the ganglia to form microfoci of infection in the skin but that these microfoci are usually eliminated (Hill & Blyth, 1976). Physiological changes in the skin, perhaps mediated by prostaglandins, occasionally allow lesions to develop (skin triggering theory). The possible role of prostaglandins in this theory was supported by the fact that the stimuli that cause reactivation of HSV were associated with elevated prostaglandin levels (Hill & Blyth, 1976). For example, u.v. light, one of the most common reactivating stimuli, produces several well-recognized changes in the skin including release of prostaglandins, particularly prostaglandin E₂ (Greaves & Sondergaard, 1970). After a single administration of prostaglandin E₂ into the ear skin of latently infected mice, skin reactions similar to those induced by u.v. light were induced and they were associated with virus reappearance (Blyth et al., 1976). A known inhibitor of prostaglandin synthesis, chlorpromazine, reduced the incidence of herpes recurrence (Bradley & Samuels, 1971; Chang, 1975). Chemical stimuli that caused reactivation of latent HSV in ganglia increased the levels of prostaglandin in the skin (Harbour et al., 1983b).

Recent studies with the inoculation of HSV on the lip or cornea have shown that HSV persists for months in the trigeminal ganglia of infected mice (Openshaw et al., 1979; Sekizawa et al., 1980). For approximately 2 weeks after virus inoculation, infectious virus can be recovered from cell-free ganglionic homogenates. After 2 weeks, infectious virus can no longer be recovered, but can be reactivated by explantation of ganglia in vitro (Openshaw et al., 1979). Using trigeminal ganglia latently infected with HSV-1, we investigated the effect of indomethacin on reactivation of HSV-1. The results showed that indomethacin strongly suppresses the reactivation of HSV-1 in the ganglia explanted in vitro.

**METHODS**

*Mice.* Female, 6- to 8-week-old BALB/c mice were obtained from the Funabashi Farm, Tokyo, Japan.

*Virus.* The F strain of HSV-1 was propagated in green monkey kidney (GMK) cells as previously described (Shimizu et al., 1976). Briefly, GMK cells were infected with HSV-1 at an m.o.i. of 0.1 p.f.u./cell and incubated in Eagle’s MEM containing 2% heat-inactivated calf serum at 37 °C for 3 days. The infected cells were disrupted by freezing and thawing three times. The lysate was centrifuged at 3000 r.p.m. for 20 min and the supernatant fluid was stored as virus stock at -90 °C until use. Infectious virus titre as determined by plaque formation in GMK cells was 1 × 10⁸ p.f.u./ml.

*Reagents.* Indomethacin, tetracaine and mepacrine were purchased from Sigma. Mefenamic acid was supplied by Sankyo Pharmaceutical Co., Tokyo, Japan. Indomethacin and mefenamic acid were dissolved in 7% NaHCO₃ (Meylon®, Otsuka Pharmaceutical Co., Tokyo, Japan) and diluted with RPMI 1640 medium. An equivalent concentration of Meylon was added to the control cultures. Tetracaine and mepacrine were dissolved in RPMI 1640 medium (Gibco). All the compounds were tested for their cytotoxicity to the tissue culture cells (R66 human embryonic lung cells) by dye exclusion and growing tests. The concentrations of the compounds used in the experiments were found to be non-cytotoxic: indomethacin, 5 × 10⁻⁴ M; mefenamic acid, 5 × 10⁻⁴ M; tetracaine, 5 × 10⁻³ M; mepacrine, 1 × 10⁻⁴ M.

*Antiserum.* Adult albino rabbits obtained from the Funabashi Farm were intravenously injected with 1 × 10⁷ p.f.u. of the F strain of HSV-1 once a week for 5 consecutive weeks. Seven days after the last injection, sera were collected and heat-inactivated at 56 °C for 30 min. The neutralization titres of the hyperimmune sera were 1:256 and 1:512 by plaque reduction tests.

*Inoculation of virus.* BALB/c mice were inoculated with HSV-1 on the lip as previously described (Price et al., 1975). Briefly, epithelial surfaces of the lower lip were abraded with an emery paper, and a drop of stock virus containing 1 × 10⁷ p.f.u./ml was placed on the abraded surface. About 10% of the infected mice died by 10 days after inoculation. Trigeminal ganglia were taken from the mice that survived for more than 25 days after inoculation, and were used as latently infected ganglia for the reactivation experiments.

*Titration of infectious virus in the ganglia.* Trigeminal ganglia were aseptically removed from the mice as previously described (Sekizawa et al., 1980). After being washed twice with RPMI 1640 medium, the ganglia were suspended in 2 ml of RPMI 1640 medium, homogenized with a glass homogenizer (Wheaton Scientific Co., N.J., U.S.A.) and centrifuged at 2000 r.p.m. for 5 min. A half ml of the supernatant fluid was placed on a human embryonic lung cell (R66 cell) monolayer in plastic Petri dishes of 3-5 cm diam. (Falcon) and incubated at 37 °C for
Inhibition of HSV reactivation

2 h. The culture fluids were removed and replaced by 2 ml of RPMI 1640 medium containing 2% heat-inactivated hyperimmune rabbit serum and 2% calf serum. The plates were cultured at 37 °C for 3 to 4 days. After being washed, the cells were stained with May–Grünwald solution (E. Merck, West Germany), and the number of plaques was counted under a stereomicroscope (Olympus Optical Co., Tokyo, Japan).

Detection of reactivation in ganglia

Reactivation of latent HSV-1 in the explanted ganglia was assessed by co-cultivation assays and ganglion homogenate assays.

Co-cultivation assay of reactivation. Trigeminal ganglia were removed aseptically from the latently infected mice and washed twice with RPMI 1640 medium. Each ganglion was placed on R66 monolayer cells grown in a plastic Petri dish (3.5 cm diam.) containing 2 ml RPMI 1640 medium with 2% foetal calf serum (FCS). Dishes were cultured at 37 °C in 5% CO₂ (Sekizawa et al., 1980). Viral plaques or c.p.e. induced by HSV-1 in R66 monolayer cells were observed every day for a week under a microscope. At the end of cultivation, monolayers were washed with 5% May–Grünwald solution and the number of plaques was counted. When any plaques, even one, appeared in the R66 monolayer, we judged that reactivation had occurred in the ganglia.

Homogenate assay of reactivation. Latently infected trigeminal ganglia were removed from mice and were cultured in plastic Petri dishes containing RPMI 1640 medium and 10% FCS (RPMI/FCS) without R66 monolayer cells at 37 °C in 5% CO₂ (Wohlenberg et al., 1979). After incubation for the desired periods, ganglia were taken out, washed three times with RPMI 1640 medium and homogenized with a glass homogenizer. Infectious virus titres in the homogenates were assessed by plaque assay using R66 cells as described above.

Assay of prostaglandins. Prostaglandin E (PGE) and prostaglandin B (PGB) were measured radioimmunologically using commercial kits (CA50I, Clinical Assays, Tokyo, Japan) as previously described (Abe et al., 1977). Briefly, PGE contained in the sample was converted to PGB by alkaline treatment (Zusman, 1972). The alkali-treated samples were further acidified to pH 3 to 4 with hydrochloric acid and then shaken with ethyl acetate. The extracted organic phase was dried to yield the residue, which was applied to a silicic acid column. PGB was eluted from the column by a mixture of benzene/ethyl acetate (60/40) according to the method of Jaffe et al. (1973). After the eluates were dried up, PGB was radioimmunologically assessed by using PGB antiserum. The natural PGB was also measured by the same procedure without alkaline treatment. The results were expressed as the total amounts of PGE and PGB per ganglion.

Statistical analysis. The significance of differences between values was examined by Fisher's exact probability test. Differences yielding P values of < 0.05 were regarded as significant.

RESULTS

Establishment of latent HSV-1 infection

Establishment of latent HSV-1 infection in the trigeminal ganglia was examined. The left trigeminal ganglia were removed from mice at appropriate intervals after inoculation with HSV-1, homogenized, and examined for the infectious virus (Fig. 1). Although no or little infectious virus was detected before day 3, virus titres of 10³ to 10⁴ p.f.u. per ganglion were detected in 70% of the tested ganglia on day 4. On days 13 and 25, infectious virus could no longer be detected.

The trigeminal ganglia removed from mice on the 25th day after infection were explanted onto R66 monolayer cells. The appearance of viral plaques on R66 monolayers was checked every day for 1 week (co-cultivation assay) (Fig. 2). On day 4, HSV-1 plaques appeared on monolayers in 23% (3/13) of the dishes. The number of dishes showing detectable plaques increased rapidly, reaching a maximum of 12 out of 13 dishes on day 6.

These results confirm the previous reports stating that inoculation of HSV-1 on the skin of mice establishes latent infection in the ganglia after about 2 weeks of a productive phase of infection (Stevens & Cook, 1971; Walz et al., 1974; Hill et al., 1975; Klein et al., 1978; Openshaw et al., 1979).

Inhibitory effect of indomethacin on the reactivation of latent HSV-1 infection

The effect of indomethacin on the reactivation of HSV-1 in the explanted ganglia was examined by using the co-cultivation assay. Latently infected ganglia were cultured on R66 monolayers in the presence or absence of 1 × 10⁻⁶ M-indomethacin. No plaques could be found in the presence of indomethacin throughout the observation period of 7 days, although viral plaques were found in more than 90% of control cultures without indomethacin. Infectious virus
with low titres (10⁠₁ or 10⁠² p.f.u./ml) was detected in only two out of 13 culture fluids containing indomethacin, although infectious virus with titres of 10⁠² to 10⁠⁴ p.f.u./ml was detected in 12 out of 13 control cultures without indomethacin (P < 0.0002) on day 6 (Fig. 3).

The effect of indomethacin on the appearance of infectious virus in the explanted ganglia was examined by the ganglion homogenate assay. This was done in order to exclude the possibility that the suppressed plaque formation and virus titres in the co-cultivation assay might be due to the inhibitory effect of indomethacin on the virus growth in R66 cells but not due to the effect on reactivation of HSV-1 in the ganglia. Latently infected ganglia were explanted into Petri dishes containing RPMI 1640 medium in the presence or absence of 5 × 10⁻⁴ M-indomethacin and cultivated at 37 °C for 7 days. The ganglia were homogenized at various intervals and tested for infectious virus using R66 cells (Table 1). Infectious virus was detected as early as 1 day after the start of incubation in 20% of the control cultures. The percentage of ganglia positive for infectious virus was 90% and virus titre reached the maximum (about 10⁴⁰ p.f.u./ganglion) on day 3 or day 4. In the indomethacin-treated group, however, infectious virus was not detected except on day 2 and day 3, when infectious virus with low titre was detected in only 10 to 15% of ganglia. These results indicate that indomethacin inhibited the reactivation of latent HSV-1 in the explanted ganglia.

Reversibility of indomethacin action

To determine whether the inhibitory effect of indomethacin on reactivation of HSV-1 in the explanted ganglia is reversible or not, the explanted ganglia were cultivated in the presence of 5 × 10⁻⁴ M-indomethacin until day 3, and washed four times with fresh medium and then recultivated in the indomethacin-free medium for the following 4 days. Table 2 shows the percentage of ganglia positive for infectious virus and the virus titre of the homogenates of the
**Inhibition of HSV reactivation**

---

**Fig. 2**

Appearance of HSV-1 plaques in the R66 monolayer cells co-cultivated with latently infected ganglia. Trigeminal ganglia removed from mice 25 days after HSV-1 infection were cultured on R66 monolayer cells in 2 ml RPMI 1640 medium containing 2% FCS at 37 °C in 5% CO₂. Appearance of viral plaques or c.p.e. in monolayer cells was observed daily for a week. At the end of cultivation, monolayers were stained with May–Grünwald solution. Values represent the percentage of 13 dishes positive for plaques or c.p.e.

**Fig. 3**

Effect of indomethacin on the reactivation of HSV-1 in the explanted ganglia as revealed by virus titres in the culture fluids of ganglion and R66 cell co-cultivation. Latently infected ganglia were cultured on R66 monolayer cells in the presence or absence of 1 x 10⁻⁴ M-indomethacin. The virus titres in the culture fluids on day 6 were then assessed by plaque assay. Values represent the virus titre in each culture fluid.

---

**Table 1. Reactivation of HSV-1 in explanted ganglia in the presence or absence of indomethacin**

<table>
<thead>
<tr>
<th>Time after incubation (days)</th>
<th>Control (without indomethacin)</th>
<th>With indomethacin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive ganglia†/no. of tested ganglia (%)</td>
<td>Averaged p.f.u. per ganglion (log₁₀)</td>
</tr>
<tr>
<td>0</td>
<td>0/10 (0)</td>
<td>&lt;0</td>
</tr>
<tr>
<td>1</td>
<td>2/10 (20)†</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>6/8 (75)§</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>9/10 (90)§</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>9/10 (90)§</td>
<td>4.0</td>
</tr>
<tr>
<td>7</td>
<td>9/10 (90)§</td>
<td>ND†</td>
</tr>
</tbody>
</table>

* 5 x 10⁻⁴ M-indomethacin was added to the medium.
† Assayed by homogenate assay of reactivation.
‡ Statistical significance was determined in the percentage of reactivation between control cultures (without indomethacin) and cultures with indomethacin.
§ Statistically significant (P < 0.0003).
† ND, Not determined.
Table 2. **Reversible action of indomethacin in suppression of appearance of infectious virus in ganglia**

<table>
<thead>
<tr>
<th>Days</th>
<th>Ganglia cultured in</th>
<th>Reactivation of HSV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of positive ganglia/no. of tested ganglia (%)</td>
</tr>
<tr>
<td>3</td>
<td>Control culture</td>
<td>9/10 (90)</td>
</tr>
<tr>
<td></td>
<td>Indomethacin culture (before removal)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>7</td>
<td>Control culture</td>
<td>9/10 (90)</td>
</tr>
<tr>
<td></td>
<td>Indomethacin culture† (after removal)</td>
<td>8/10 (80)</td>
</tr>
</tbody>
</table>

* Assayed by homogenate assay of reactivation.
† Ganglia were cultured in the presence of 5 × 10⁻⁴ M-indomethacin for 3 days and then cultured in the indomethacin-free medium for the following 4 days.
‡ ND, Not determined.

Table 3. **Inhibition by indomethacin of reactivation of HSV-1 in the ganglia: effect of time of addition**

<table>
<thead>
<tr>
<th>Time of addition* of indomethacin (h after beginning of incubation)</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition (control)</td>
<td>7/10 (70)</td>
<td>8/10 (80)</td>
</tr>
<tr>
<td>0</td>
<td>0/10 (0)†</td>
<td>1/16 (6)§</td>
</tr>
<tr>
<td>4</td>
<td>6/18 (30)§</td>
<td>4/20 (20)‡</td>
</tr>
<tr>
<td>12</td>
<td>6/10 (60)§</td>
<td>8/10 (80)§</td>
</tr>
<tr>
<td>24</td>
<td>5/10 (50)§</td>
<td>ND¶</td>
</tr>
<tr>
<td>48</td>
<td>6/10 (60)§</td>
<td>7/10 (70)¶</td>
</tr>
</tbody>
</table>

* 5 × 10⁻⁴ M-indomethacin was added at different times after the beginning of incubation.
† Statistical significance was determined in the percentage of reactivation between control cultures and cultures with indomethacin.
‡ Statistically significant, 0.001 < P < 0.003.
§ Statistically significant, P < 0.0002.
¶ Not significant.
¶ ND, Not determined.

Explanted ganglia before and after removal of indomethacin. After removal of indomethacin, reactivation occurred in the ganglia that had been cultivated in the presence of indomethacin for 3 days. These results suggest that the inhibitory effect of indomethacin is reversible and is not due to a cytotoxic effect on the ganglionic cells.

**Effect of the time of indomethacin addition on the appearance of infectious virus in the ganglia**

The effect of the time of indomethacin addition on reactivation of HSV-1 in the explanted ganglia was examined. Indomethacin (5 × 10⁻⁴ M) was added to the ganglia cultures at the initiation time of incubation or at different times after the beginning of incubation. All ganglia were cultured at 37 °C for 4 days, taken out, homogenized and then examined for the presence of infectious virus (Table 3). The appearance of infectious virus in the ganglia was almost completely suppressed when indomethacin was added to the culture at the initiation time of cultivation and was partially inhibited when it was added at 4 h. The appearance of infectious virus was not inhibited in the cultures to which indomethacin was added at 12 h or later.

Overall, these results indicate that indomethacin acted on the early process of the appearance of infectious virus, resulting in inhibition of virus growth in the ganglia.
Table 4. *Inhibition by indomethacin of prostaglandin synthesis and HSV-1 reactivation*

<table>
<thead>
<tr>
<th>Ganglia*</th>
<th>Indomethacin</th>
<th>Prostaglandins† (pg/ganglion)</th>
<th>Reactivation of HSV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Culture fluid</td>
<td>Homogenate</td>
</tr>
<tr>
<td>Non-infected</td>
<td>-</td>
<td>1649</td>
<td>178</td>
</tr>
<tr>
<td>Latently infected</td>
<td>-</td>
<td>1221</td>
<td>114</td>
</tr>
<tr>
<td>Latently infected +</td>
<td>+</td>
<td>64</td>
<td>0</td>
</tr>
</tbody>
</table>

* Left trigeminal ganglia were removed from 16 latently infected mice. Eight ganglia were cultured for 4 days in the presence or absence of 5 × 10⁻⁴ M-indomethacin. As a control, eight trigeminal ganglia were removed from uninfected mice and cultured for 4 days in the absence of indomethacin.
† The pooled culture fluids or homogenates of eight ganglia were assayed. The results are expressed as a mean value per one ganglion.

Table 5. *Inhibition of reactivation of HSV-1 by other inhibitors of prostaglandin synthesis*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Dose (Μ)</th>
<th>No. of positive ganglia/no. of tested ganglia (%)</th>
<th>Virus titre in the homogenates (log₁₀ p.f.u./ganglion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1 Control</td>
<td>-</td>
<td>11/14 (79)</td>
<td>4:6</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>5 x 10⁻³</td>
<td>0/10 (0)</td>
<td>&lt;0</td>
</tr>
<tr>
<td>Expt. 2 Control</td>
<td>-</td>
<td>9/12 (70)</td>
<td>4:3</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>5 x 10⁻³</td>
<td>0/13 (0)</td>
<td>&lt;0</td>
</tr>
<tr>
<td>Mepacrine</td>
<td>1 x 10⁻⁴</td>
<td>0/13 (0)</td>
<td>&lt;0</td>
</tr>
</tbody>
</table>

* Latently infected ganglia were cultivated in the presence or absence of the reagents for 4 days at 37 °C and assayed for infectious virus in their homogenates.

*Correlation between suppression of HSV-1 reactivation and inhibition of prostaglandin synthesis by indomethacin*

Next, to determine whether there is any correlation between the suppression of HSV-1 reactivation by indomethacin in the explanted ganglia and the inhibition of prostaglandin synthesis, the amounts of prostaglandins in the culture fluids and in the homogenates of ganglia were assayed (Table 4). Latently infected ganglia were cultivated in vitro at 37 °C for 4 days in the presence or absence of 5 × 10⁻⁴ M-indomethacin. 1221 pg/ganglion of prostaglandin was detected in the culture fluids and 114 pg/ganglion was detected in the homogenates, when latently infected ganglia were cultivated in the absence of indomethacin. These values were similar to levels detected in uninfected ganglia. In contrast, much lower amounts of prostaglandin were detected in the culture fluids (60 pg) and in homogenates (0 pg) of latently infected ganglia when ganglia were cultivated with indomethacin. Inhibition of prostaglandin synthesis and complete suppression of HSV-1 reactivation were observed concomitantly on day 4 in the ganglia cultivated in the presence of indomethacin.

*Inhibition of HSV-1 reactivation by other inhibitors of prostaglandin synthesis*

Possible involvement of prostaglandin synthesis in HSV-1 reactivation in the explanted ganglia was also examined using other reagents capable of inhibiting prostaglandin synthesis: mefenamic acid, an inhibitor of cyclooxygenase, and tetracaine and mepacrine, inhibitors of phospholipase A₂. Latently infected ganglia were cultivated at 37 °C for 4 days in the presence or absence of 5 × 10⁻⁴ M-mefenamic acid, 5 × 10⁻³ M-tetracaine or 1 × 10⁻⁴ M-mepacrine and then assayed for infectious virus in the homogenates (Table 5). All of the reagents inhibited the reactivation of HSV-1, as did indomethacin.

After removal of the agents, reactivation of latent HSV-1 occurred in the ganglia which had been cultured with mefenamic acid or tetracaine (data not shown).
DISCUSSION

In the present work we have shown that indomethacin inhibits the reactivation of HSV-1 in latently infected ganglia explanted in vitro. This conclusion is based on the following results: (i) plaque formation in R66 cell monolayers co-cultivated with latently infected ganglia was inhibited by indomethacin; (ii) the appearance of infectious virus in the explanted ganglia was also inhibited by indomethacin; (iii) after removal of indomethacin from the ganglia cultures, reactivation of latent HSV-1 occurred and the virus titre reached levels found in the indomethacin-free cultures, suggesting that the effect of indomethacin may not be due to a cytotoxic effect on the ganglionic cells; (iv) the effect of indomethacin was only seen when it was added within a short time after explantation, suggesting that indomethacin might affect some mechanisms of the early phase of viral reactivation. We have also shown that the inhibition of HSV reactivation by indomethacin was associated with an almost complete suppression of prostaglandin (PGE and PGB) synthesis in the explanted ganglia (Ferreira et al., 1971; Yorio & Bentley, 1978). Other inhibitors of arachidonic acid metabolism such as tetracaine (Rubin & Laychock, 1978) and mepacrine (Flower & Blackwell, 1976; Vargaftig, 1977), which are phospholipase A2 inhibitors, and mefenamic acid (Flower & Vane, 1974; Yorio & Bentley, 1978), which is an inhibitor of cyclooxygenase, inhibited viral reactivation in the explanted ganglia.

The possible role of prostaglandins in HSV reactivation was first suggested by Hill & Blyth (1976), based on the fact that the stimuli provoking a recurrence of HSV infection were associated with elevated prostaglandin levels. To explain reactivation of HSV, they proposed a hypothesis that HSV is often released from the ganglia to form microfoci of infection in the skin but that these microfoci are usually eliminated. Physiological changes in the skin, perhaps induced by prostaglandins, occasionally allow lesions to develop (skin triggering theory). Recently, they also suggested that some stimuli can provide both the ganglion trigger and the skin trigger to induce clinical recurrent herpes (the ganglion and skin trigger theory) (Hill, 1981; Harbour et al., 1983b). According to this theory, the virus would be reactivated in the ganglion by the stimulus to the skin, and then travel to the skin. In the skin, physiological changes induced by the original stimulus allow lesions to develop. Our results suggest a possible role of prostaglandins in reactivation of HSV-1 in the ganglia, and may support the ganglion and skin trigger theory, although the results do not provide any evidence concerning the development of lesions in the skin.

The effects of prostaglandins on the replication of HSV in conventional tissue culture have been reported by some investigators. Harbour et al. (1978) reported that PGE increased the size of the plaques and the yield of HSV, and that inhibitors of prostaglandin synthesis decreased them. They also suggested that increased adhesion between cells containing HSV and uninfected cells by PGE may be the cause (Harbour et al., 1983a). Newton (1979, 1982) showed that inhibitors of prostaglandin synthesis, i.e. indomethacin, aspirin, mefenamic acid and flufenamic acid inhibited HSV replication in conventional tissue cultures. Treatment of HSV-infected L cells with indomethacin, mefenamic acid and flufenamic acid caused a considerable reduction in the production of viral DNA. These effects were seen only when the reagents were added within the first hour of infection. No effect of the reagents on DNA synthesis or cell division could be observed in uninfected cells, suggesting that these reagents may affect some metabolic system of host cells that is required by the virus. The suppressive effect on the reactivation of latent HSV-1 in the explanted ganglia was also seen only when indomethacin was added in the very early stages before active viral DNA replication occurred. This result may suggest that indomethacin inhibits reactivation by suppressing the replication of viral DNA. It remains, however, undetermined whether the products of arachidonic acid metabolism including prostaglandins are involved directly in the reactivation of latent virus or that the inhibitory effects of indomethacin and other reagents are due to some of their other functions. HSV reactivation in the explanted ganglia was partially inhibited by $10^{-5}$ M- but not by $10^{-6}$ or $10^{-9}$ M-indomethacin (data not shown), although indomethacin is normally reported to be effective in inhibition of prostaglandin synthesis in the range $10^{-9}$ M to $10^{-8}$ M (Thomas et al., 1974; Newton, 1982). Newton also showed that in conventional tissue culture the concentration
Inhibition of HSV reactivation

at which indomethacin is active on the replication of HSV is from $10^{-5}$ to $10^{-3}$ M (Newton, 1979). This may be due to the instability of these compounds in tissue culture conditions, and their binding to serum proteins.

In ganglia having a latent HSV infection, viral DNA has been detected by hybridization in situ using a radioactive complementary RNA probe made from HSV DNA (Puga et al., 1978). The state of the viral genome during latency, however, is not clearly known. Some reports favour a complete block in the transcription of the viral genome (Puga et al., 1978). Other studies report that HSV-specific ribonucleic acid (Galloway et al., 1979) and an immediate early HSV polypeptide can be found in the ganglia, suggesting that some transcription of the viral genome occurs in the ganglia (Green et al., 1981a). If arachidonic acid metabolism plays a role in reactivation of HSV in the latently infected ganglia, it may be involved in early stages of progeny virus synthesis such as replication of viral DNA or transcription of the viral genome. The effects of inhibitors of prostaglandin synthesis on HSV reactivation provide tools useful for elucidating the state of viral genome during latency and the mechanisms of its reactivation.

We thank Dr K. Abe and Dr K. Sato, 2nd Department of Internal Medicine, Tohoku University School of Medicine, for assay of prostaglandins. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES


(Received 13 February 1984)