Interaction of Polylysine with the Cellular Receptor for Herpes Simplex Virus Type 1

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SUMMARY

We earlier reported that neomycin blocked reversibly the binding of herpes simplex virus type 1 (HSV-1) to the receptor of BHK cells, while the binding of HSV-2 to the receptor was unaffected. We could not determine whether the effect was on the virus particle, the receptor, or both. We have now tested several other cationic substances, and report that polylysine (and polyarginine) block the binding of HSV-1 to the receptor by interfering with the cellular receptor function; higher molecular weight polylysines were more potent than those of lower molecular weight. Polylysine and neomycin showed additive effects. In vitro, polylysine showed the same strong binding to the plasma membrane phosphoinositides as did neomycin. Together these data suggest that the drugs may have a common target in the cell membrane. The HSV-1 and HSV-2 virus particles were unaffected by the drugs, as was the cellular HSV-2 receptor.

INTRODUCTION

Interactions between herpes simplex virus (HSV) and its cellular receptors are poorly understood. Evidence has been presented that the cellular receptor is glycoprotein in nature (Mohanty & Rosenthal, 1986; Zeigler & Pozos, 1981) and that HSV-1 and HSV-2 have separate receptors on the host cell's surface (Addison et al., 1984; Vahlne et al., 1979). We showed previously that the inositol lipid-complexing agent, neomycin, specifically inhibited binding of HSV-1 to the cellular receptor, while HSV-2 binding was unaffected (Langeland et al., 1986b). Neomycin was chosen since HSV-1 induces altered cellular inositol lipid turnover (Langeland et al., 1986a). Neomycin has been used extensively as an inhibitor of phosphoinositide-mediated events in eukaryotic cells; it inhibits thrombin-induced cell growth (Carney et al., 1985), Ca2+-dependent secretion in mast cells (Cockcroft & Gomperts, 1985), inositol phosphate release from erythrocyte ghosts (Downes & Michell, 1981), and Ca2+-dependent exocytosis in sea urchin eggs (Whitaker & Aitchison, 1985). In kidney brush border membranes, the cellular binding site for the aminoglycoside antibiotics has been determined to be a phosphoinositide (Sastrasinh et al., 1982). Recently, nuclear magnetic resonance studies have revealed very rapid complexing kinetics between neomycin and phosphatidylinositol 4,5-bisphosphate (PIP2), indicating appropriate spacing between the anionic and cationic groups of the two molecules (Reid & Gajjar, 1987).

From the previous results on the effects of neomycin, we could not determine whether it affected the virus particle or the cellular receptor (Langeland et al., 1987). We therefore searched for other agents which might have similar effects. We also wanted to investigate whether it was the cationic properties of neomycin, or more specific properties, which caused its antiviral effect. Polylysine is a polycation which has been shown to bind to the aminoglycoside binding site in kidney cells (Williams et al., 1986). Therefore it may have some of the same effects as neomycin. An inhibitory effect of polylysine on certain viral infections (tobacco mosaic virus) has been reported (Stahmann et al., 1950), while other viruses (potato virus X) seem to be unaffected (Prakash & Foxe, 1985). Polylysine has been shown to have diverse effects on various...
Effect of different poly-amino acids on HSV-1 infection

Polylysine was first tested at concentrations at which the total cationic charge was similar to that of aminoglycosides used in earlier experiments in which HSV-1 infection was inhibited efficiently (Langeland et al., 1987). A small poly-L-lysine ($M_r$ 3700), with 18 amino groups, was bound to beaded agarose was purchased from Sigma.
Polylysine effect on HSV-1 infection

Fig. 1. Effect of different polylysines on HSV-1 plaque formation. Poly-L-lysine $M_r$ 3700 (△), poly-L-lysine $M_r$ 17300 (□), poly-D-lysine $M_r$ 13800 (●) or poly-L-lysine $M_r$ 52000 (○) were added to the cells at the indicated concentrations 10 min before the addition of virus. The agents were present during the adsorption period (1 h), then removed by change of medium. Plaque formation was recorded after 48 h. One-hundred % plaque formation was defined as the number of plaques obtained without polylysine additions. Data were determined from triplicate samples and similar results were reproduced in four individual experiments.

Fig. 2. HSV-1 infection with addition of polylysine at different times before or after infection. Ten $\mu$M-polylysine ($M_r$ 3700) was added at the indicated times before or after infection (indicated by the arrow). Plaque formation without polylysine addition (100%) is indicated (open circle). Conditions otherwise as indicated for Fig. 1.

used initially. This polypeptide, at a concentration of 1 mM, has an approximately equivalent cationic charge to 3 mM-neomycin, which was fully inhibitory on infection. However, this polylysine was toxic to the cells at millimolar concentrations. Larger polymers of polylysine were toxic at lower concentrations and polylysines of $M_r$ 50000 were toxic in the micromolar range. Doses which were effective against virus infection also varied with the $M_r$ of the individual polylysines; $M_r$ 52000 was effective at 10 $\mu$M, $M_r$ 17000 at 100 $\mu$M, and $M_r$ 3700 at 5 $\mu$M (Fig. 1). Poly-D-lysine was as effective as poly-L-lysine of corresponding $M_r$.

The time of addition of polylysine was also important. As with neomycin (Langeland et al., 1986b), polylysine had to be present at the time of infection in order to be fully effective (Fig. 2). When polylysine was present at inhibitory concentrations (10 $\mu$M, $M_r$ 3700) from 10 min before infection and throughout the adsorption period, 95 % of the plaque formation was inhibited. Addition of polylysine at 60 min post-infection, however, had no effect on plaque formation. The kinetics of virus adsorption and penetration, under the experimental conditions used, has been documented (Fig. 5 in Langeland et al., 1987) and the results given in Fig. 2 are consistent with this, suggesting that polylysine interferes with very early stages of HSV-1 infection.

Additive effect of neomycin and polylysine

Neomycin and polylysine were present simultaneously so that the combined effect was 50% plaque reduction. All doses were performed in triplicate dishes. When the doses giving 50% plaque reduction were combined graphically, the slope was a straight line (Fig. 3). Thus, the effects of the polycations were not synergistic or antagonistic, but additive.

Poly-L-arginine, poly-L-histidine and poly-L-leucine were also tested for antiviral activity. While the leucine and histidine polymers did not show any inhibitory effect on HSV-1 infection, poly-L-arginine and poly-L-lysine of similar sizes were almost equally efficient at the various concentrations (Fig. 4).

Pre-treatment of virus or cells with polylysine

In order to discriminate between the possibilities that either the virus or the cell was affected by polylysine, experiments were performed in which either the cells or the virus were pre-treated
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Fig. 3. Effect on HSV-1 infection by interaction between polylysine and neomycin. Polylysine ($M_r$ 3700) and neomycin, at various concentrations, were added simultaneously 10 min before infection. The drug combinations that gave 50% plaque reduction are shown. All data are the mean values of triplicates.

Fig. 4. Effects of various poly-amino acids on HSV-1 plaque formation. Polylysine $M_r$ 52000 (○), polyarginine $M_r$ 40000 (●), polyhistidine $M_r$ 10000 (△) or polyleucine $M_r$ 14000 (▲) were added at the indicated concentrations 10 min before infection. Conditions otherwise as for Fig. 1.

Fig. 5. Effect of polylysine pre-treatment of cells or virus. Either the cells (3) or virus (2) were treated with 10 μM polylysine ($M_r$ 3700) for 30 min before infection. Before infection the polylysine-containing medium on the cells was replaced and the pre-treated virus was diluted 1:1000 so that only either virus or cells were exposed to effective doses of polylysine. Control experiments were performed without polylysine (1) or with polylysine present from 10 min before infection and throughout the adsorption period (4).

with polylysine (Fig. 5). Before infection, polylysine was incubated for 30 min with either the virus or the host. Prior to infection polylysine was removed from the pre-treated cells, and pre-treated virus was diluted 1:1000 before infection of the cells. The results (Fig. 5) clearly show that pre-treatment of virus did not affect infection, whereas pre-treatment of the cells was almost as effective as the presence of polylysine during the adsorption period.

Site of action of polylysine

Experiments were performed to determine whether the effect of polylysine was on receptor binding, on the process of internalization or on internalized virus. In all cases, adsorption of virus was performed at 4 °C for 1 h; the cells were then washed and internalization was permitted by further incubation at 37 °C. Polylysine ($M_r$ 3700, 10 μM) was added either before virus, after the adsorption period but before internalization, or after internalization had occurred. As expected, polylysine was effective when added before infection and ineffective when virus had become internalized (Table 1). There was a minor effect on bound virus, but markedly less than that on unbound virus.

We also studied the binding of radioactive virus, comparing the effects of polylysine and neomycin on $^{35}$S-labelled HSV-1 and HSV-2 binding to the cells (Table 2). Plaque formation of HSV-1 was 85% inhibited at 0.1 μM-polylysine ($M_r$ 17000), while binding of radioactive HSV-1 was inhibited by 71%. Corresponding data were obtained for neomycin. Polylysines of lower $M_r$ were less effective in inhibiting HSV-1 binding (data not shown). HSV-2 binding and infection were almost totally unaffected by both neomycin and polylysine. For both HSV-1 and HSV-2, approx. 30% of the input radiolabelled virus became bound to the cells in the control dishes without drugs added. It should be noted that the input m.o.i. in the plaque assay was 1 x 10⁴ to 5 x 10⁴ times less than the input m.o.i. in the radiolabelling experiments. However, even this input was far below receptor saturation levels.
**Polylysine effect on HSV-1 infection**

Table 1. Effect of polylysine and neomycin at various stages of HSV-1 infection*

<table>
<thead>
<tr>
<th>Drug added to virus</th>
<th>Neomycin (Plaque formation (%))</th>
<th>Polylysine (Plaque formation (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbound</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Bound</td>
<td>92</td>
<td>83</td>
</tr>
<tr>
<td>Internalized</td>
<td>103</td>
<td>98</td>
</tr>
</tbody>
</table>

* Experiments performed at 4°C up to internalization. One-hundred % plaque formation corresponds to plaque number on untreated cells. Unbound virus, drug added to the cells before infection; bound virus, drug added after 1 h adsorption at 4°C and removal of unbound virus; internalized virus, drug added after adsorption and 30 min at 37°C to allow internalization of virus.

Table 2. Effect of neomycin and polylysine on binding of radiolabelled HSV-1 and HSV-2 and on plaque formation*

<table>
<thead>
<tr>
<th>Additions before infection</th>
<th>Plaque formation (%)</th>
<th>Radioactivity bound (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSV-1</td>
<td>HSV-2</td>
</tr>
<tr>
<td>No additions</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5 mM Neomycin</td>
<td>16</td>
<td>94</td>
</tr>
<tr>
<td>0.1 μM Polylysine</td>
<td>15</td>
<td>95</td>
</tr>
</tbody>
</table>

* One-hundred % plaque formation corresponds to plaque number without drugs added. Polylysine (Mr, 17300) or neomycin was added 10 min before infection and remained in the medium throughout the adsorption period. One-hundred % radioactivity bound, radiolabel bound without drugs added. For HSV-1 this averaged 2600 c.p.m./dish, and for HSV-2 it averaged 1450 c.p.m./dish. Experiments were performed at 37°C, incubation periods for labelled virus were 1 h.

In vitro study on polylysine’s ability to bind membrane phospholipids

Since the effect of polylysine was on the host cells and could be mediated through binding to phospholipids in the cell membrane (Bashford et al., 1986), it was of interest to study the ability of polylysine to bind to various phospholipids. Cells were pre-labelled with 32P, and phospholipids were extracted as described in Methods. They were then eluted from a polylysine column with increasing ammonium formate concentrations. The phospholipids that did not bind eluted with the sample solvent, while those which were increasingly strongly bound eluted with increasing concentrations of ammonium formate. Fig. 6 shows that only the acidic phospholipids bound to the column, and the polyphosphoinositides more strongly than any of the others. The binding properties paralleled those found for neomycin, which bound the polyphosphoinositides very strongly as also shown by others (Palmer, 1981).

**DISCUSSION**

Based on our previous observations that neomycin interfered with the receptor binding of HSV-1 (Langeland et al., 1987), we tested other cationic substances, such as polylysine, spermine and glucosamine. Preliminary data showed that only polylysine had any effect on infection (Langeland et al., 1987). We have now studied, more extensively, the effect of different polylysines and other poly-amino acids on HSV infection.

Polylysines inhibit infection of HSV-1 at much lower concentrations than neomycin, both when molarity and total cationic charge are considered. The potency is dependent upon the size of the polymer. Thus, polylysine of $M_r$ 17300 is approx. 10 times more potent (per mg) than polylysine of $M_r$ 3700, and polylysine of $M_r$ 52,000 is approx. three times more potent (per mg) than that of $M_r$ 17300. Thus, it cannot only be the number of ionized amino groups which...
Fig. 6. Binding of membrane phospholipids to polylysine and neomycin columns. Thin-layer chromatograms of $^{32}$P-labelled phospholipids eluted from polylysine (lanes 1 to 9) or neomycin (lanes 10 to 14) columns. Lane 1 was a sample of the total phospholipid mixture which was added to the column. Lane 2 was the eluate from C:M 1:1. Lane 3 was the eluate from C:M:formic acid 5:10:2. Lanes 4 to 14 were eluates from addition of the indicated molar concentrations of ammonium formate. The individual phospholipids are indicated to the left in the figure: PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; PS, phosphatidyl serine; PI, phosphatidyl inositol; PIP, phosphatidylinositol monophosphate; PIP$_2$, phosphatidylinositol bisphosphate. Procedures otherwise as described in Methods.
determines the potency of the drug. Nevertheless, the effect seems to be related to the fact that the substances are cationic. Of the poly-amino acids tested, only polylysine and polyarginine affected HSV infection. Polyleucine as expected was not effective, since it has no cationic charge. Polyhistidine was not effective either in spite of its cationic imidazole group; at the neutral pH used, the imidazole NH group is only weakly ionized, which may partly explain the lack of effect. The doses tested, however, were high enough to compensate for the weak ionization; this agrees with the assumption that cationic charge alone is not sufficient to explain the inhibitory effects on HSV-1 infection. The lack of effect of glucosamine and spermine also support the idea that cationic charge alone is insufficient to prevent HSV-1 infection: it appears that a specific spacing of the cationic groups is necessary for this. As was the case with neomycin, polylysine had to be present at the time of infection in order to be effective. Unlike neomycin, however, polylysine was effective when used to pre-treat the cells. This strongly suggests an interaction with the cellular receptor or the cell membrane which thus inhibits internalization of the virus. The data on the effect of polylysine on unbound, bound and internalized virus suggest that receptor binding was probably inhibited (Table 1). The slight effect on receptor-bound (but not internalized) virus may indicate either a competition with virus on receptor binding, or an additional post-receptor but pre-internalization effect of polylysine. However, the data presented in Table 2 suggest that the effect was mainly on receptor binding, since polylysine inhibited binding to the same extent as neomycin.

Two lines of evidence indicate that polylysine and neomycin share a common target. First, both inhibit HSV-1 infection and have no effect on HSV-2 infection (Table 2). Second, the effects of the two drugs were additive (Fig. 3). Though suggestive of a common mechanism of action, this is not evidence that the two drugs act by binding to phosphoinositides, which we suggested to be a possible mechanism for the action of neomycin (Langeland et al., 1986b). While there is substantial evidence that a phosphoinositide, PIP2, is the cellular binding site for aminoglycoside antibiotics in eukaryotic cells (Shacht, 1979; Sastrasinh et al., 1982; Tachibana et al., 1986), no such evidence has been provided for polylysine. On the contrary, many other cellular and membrane effects have been documented (Leiderman et al., 1985; Le Petit et al., 1986; Bashford et al., 1986). Therefore, we tested the ability of polylysine to bind membrane phospholipids in vitro in order to determine whether a common target on a phosphoinositide was a possible cause of the two drugs’ common action on HSV-1 infection. We were able to demonstrate that they had very similar binding properties (Fig. 6). This does not prove that such specific binding occurs in vivo, although evidence in support of this theory has been obtained in other laboratories. Williams et al. (1986) have shown that simultaneous administration of cationic poly-amino acids and aminoglycosides to rats protected renal membranes from the toxic effects of the aminoglycosides. Furthermore, the agents competed for the same binding site in studies in vitro but the antimicrobial activity of the aminoglycosides was unaffected by the presence of poly-amino acids. It has also been shown that acetylcholinesterase, which is known to be anchored to the plasma membrane via an inositol lipid (Low, 1987), was inhibited by polylysine (Lin et al., 1977). Our results and those of Williams et al. (1986) indicate that polylysines are effective at much lower concentrations than neomycin (and other aminoglycosides). This may in part be explained by a rapid internalization of polylysine (Shen et al., 1985) but not of neomycin. Consistent with this, the effect of neomycin is greatly increased in permeabilized cells (Cockcroft & Gomperts, 1985). This would also explain the apparent discrepancy in our results between the potencies of neomycin and polylysine in vitro (Fig. 6) and in vivo (Fig. 3).

In conclusion, we have shown that polylysine inhibits HSV-1 infection by affecting its binding to the cellular receptor. The results also indicate that the target is the cellular receptor itself, and not the virus particle. Furthermore, the similar properties and the additive effects of neomycin and polylysine suggest a common site of action and it appears that the spacing of the cationic groups is essential for the antiviral effect. In vitro experiments suggest that this common target may be a phosphoinositide, but this remains to be confirmed.

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REFERENCES


Polylysine effect on HSV-1 infection


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