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EVIDENCE THAT NEOMYCIN INHIBITS HSV 1 INFECTION OF BHK CELLS

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The effect of neomycin on the Herpes Simplex Virus (HSV) type 1 and 2 infection of baby hamster kidney cells was studied. Neomycin concentrations of 3 mM or more caused a more than 90% inhibition of HSV 1 proliferation, while it had no effect on HSV 2 proliferation, measured as plaque-forming units. Furthermore, neomycin must be present at the time of infection in order to exert full effect, addition 1 hour postinfection was comparable to untreated cells. This indicates that neomycin may specifically interfere with very early stages of HSV 1 infection.

Neomycin is an antibiotic which exerts its antibacterial effect by binding to the 30S ribosomal subunit of prokaryotic cells and thereby inhibiting bacterial protein synthesis (1). However, the effect on eukaryotic cells, and the main reason for its oto- and nephro-toxic effect, has been linked to neomycin's ability to bind specifically to polyphosphoinositides (2). It has also been shown that neomycin inhibits polyphosphoinositide-mediated cellular processes (3). Lately, evidence has been given that inositol-containing lipids may constitute an anchoring domain for certain plasma membrane proteins (4,5). Little is known about the cellular receptors of HSV 1 and HSV 2. Published data suggest that HSV 1 and 2 have separate receptors on baby hamster kidney (BHK) cells (6,7). Here we show that neomycin specifically inhibits HSV 1 infection of BHK cells, as opposed to HSV 2 infection which is unaffected.

MATERIALS AND METHODS

Materials. Eagle's minimum essential medium (EMEM) and newborn calf (NBC) serum were purchased from Flow Laboratories (Irvine, Ayrshire, Scotland). Neomycin sulfate (90-95% neomycin B, remainder neomycin C) was purchased from Sigma Chemical Co. (St.Louis, MO, USA). Cell culture dishes were from Nunc (Roskilde, Denmark).Fluorescent

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isothiocyanate- conjugated rabbit anti-mouse immunoglobulin (DAKO F232) was purchased from DAKOPATTS a/s (Copenhagen, Denmark). The antibody MA 1147 was a gift from dr. V. Preston, MRC Virology unit, Glasgow, Scotland.

Cell culture. BHK 21 clone 13 cells were grown until confluence in 32 mm culture dishes with EMEM supplemented with 10% NBC serum. Experiments were then performed as described.

Virus stocks and antibodies. The virus strains used were HSV 1 strain 17syn+ and HSV 2 strain HG52. The monoclonal antibody used was MA 1147 (6).

Virus titration experiments. Neomycin sulfate was dissolved in complete EMEM, pH of the solution adjusted to 7.40, and the drug was added to the cells at the indicated times and concentrations. Cells were then infected with virus in amounts yielding 30-50 plaque forming units per dish. Incubations were then continued for 48 hours at 37°C, at which time plaque formation was recorded.

Indirect immunofluorescence. Cells were grown on glass coverslips in culture dishes. neomycin was added as described above, infection was carried out at a multiplicity of 5 plaque forming units per cell. 6 hours postinfection cells were fixed in methanol at -20°C. Further immunofluorescence labelling procedure was carried out essentially as described by Addison et al. (6). The monoclonal antibody used, MA 1147, was specific for the major DNA binding protein Vmw136 (ICP8) of HSV 1 and the equivalent polypeptide of HSV2.

RESULTS

HSV 2 titre was not significantly affected by the presence of neomycin concentrations up to 7.5 mM, as shown in Fig.1. On the other hand,

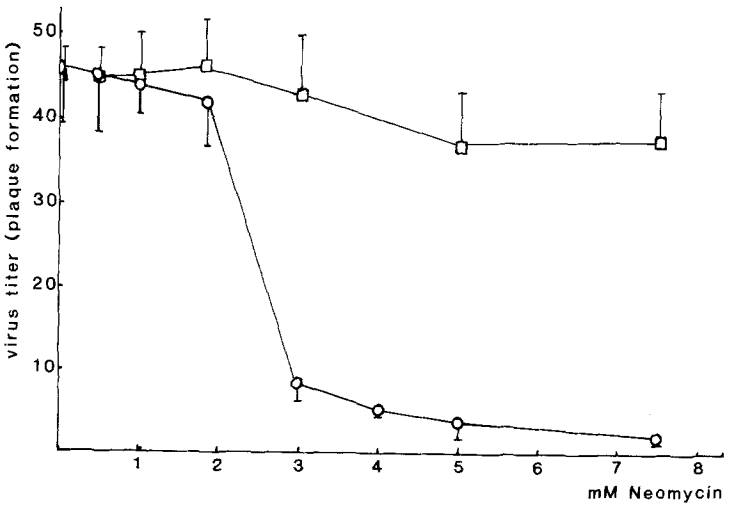

FIGURE 1. Effect of increasing neomycin concentrations on plaque formation of HSV 1 and HSV 2. BHK cells were incubated with the indicated final concentrations of neomycin 30 min prior to the addition of virus. HSV 1 infection-○-. HSV 2 infection-□-. Equal amounts of infective virus were added to all dishes. All dishes were incubated for 48 hours at 37°C, plaques were then counted. Data are given as means ± S.E.M. for three separate dishes.

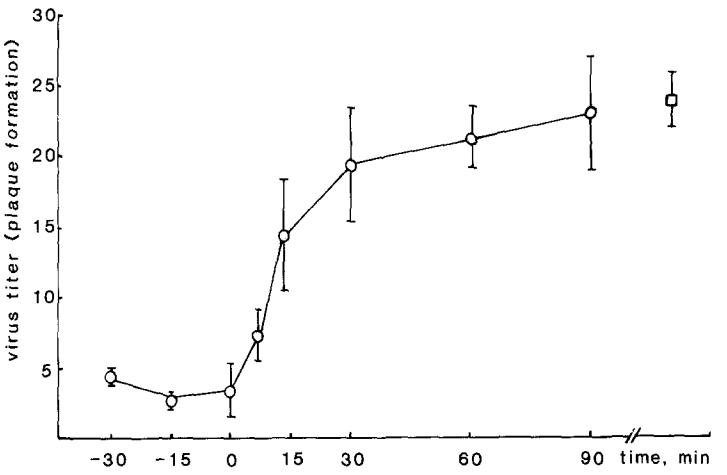

FIGURE 2. Time course of neomycin effect on HSV 1 plaque formation. Neomycin at a final concentration of 5 mM was added at the indicated times before and after infection. Further incubation as indicated in Fig. 1. The square at the right in the figure indicate parallel incubations without neomycin added. Data are means ± S.E.M. for three separate dishes.

neomycin concentrations of 3 mM and above, inhibited HSV 1 formation by more than 90% (Fig.1). Thus it seems that neomycin at these concentrations was a specific inhibitor of HSV 1 production. In the experiment described in Fig.1, neomycin was added 30 min before infection. Fig.2 shows that the neomycin effect is strongly dependent on the time before or after infection at which neomycin was added. Addition of 5 mM neomycin up to the time of infection yielded maximal effect. When neomycin was added after the addition of virus, the inhibitory effect on virus production was quickly lost. At 90 min postinfection the virus titre was not affected by neomycin even though neomycin was present from this time and until plaques were counted 48 hours postinfection.

The effect of neomycin on virus infection is also illustrated in Fig.3 where the production of virus-specific polypeptides is illustrated using a monoclonal antibody specific for the major viral DNA-binding protein ICP8. 5mM neomycin (Fig.3 B) completely abolished the production of this protein. Other antibodies were also tested, with similar results (data not shown), indicating that the overall

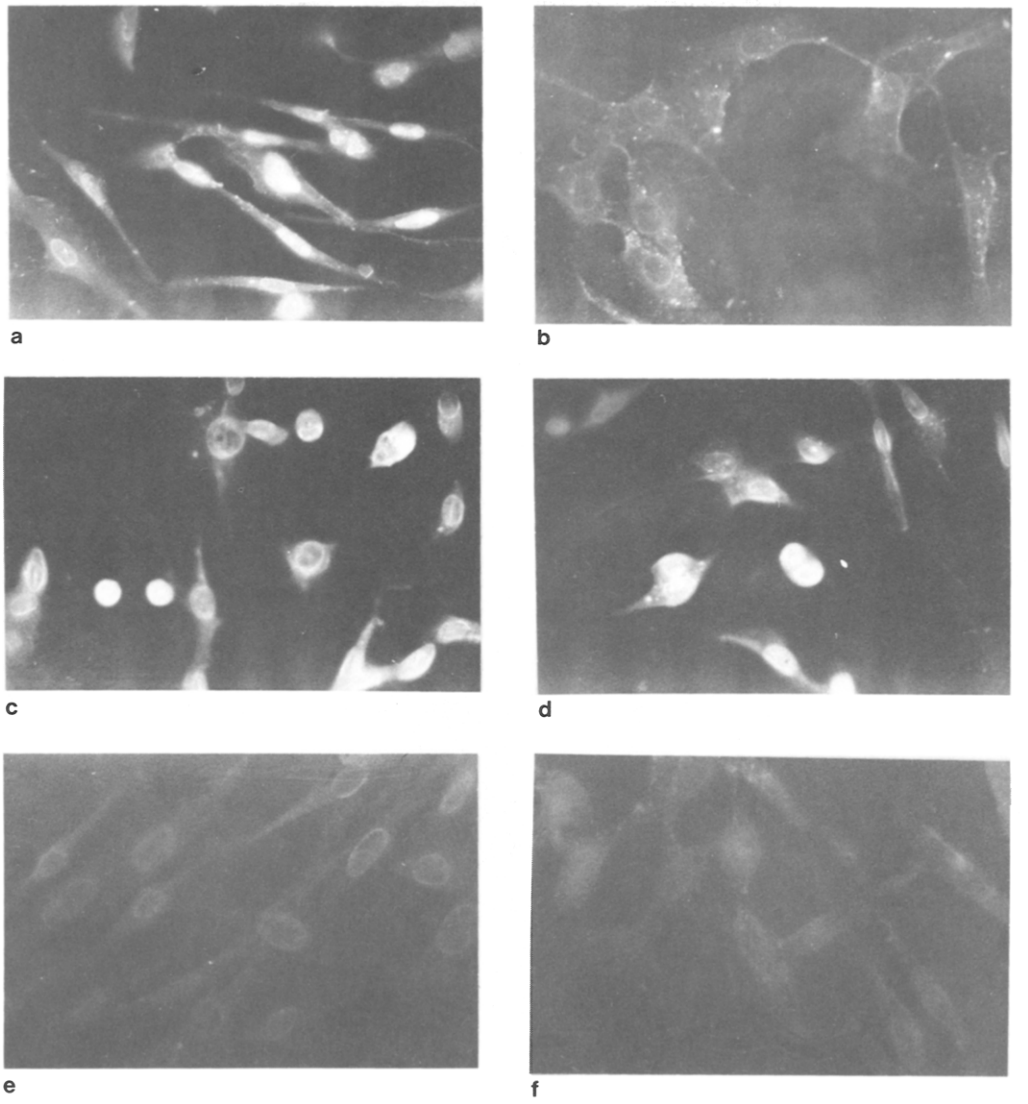

FIGURE 3. Indirect immunofluorescense studies of BHK cells infected with HSV 1 and HSV 2. Cells were either pretreated with 5 mM neomycin (b and d) or no pretreatment (a, c, e and f). Cells were incubated with the monoclonal antibody MA1147, except for f, which served as a control. a. HSV 1 infected cells without neomycin. b. HSV 1 infected cells with 5 mM neomycin. c. HSV 2 infected cells without neomycin. d. HSV 2 infected cells incubated with 5 mM neomycin. e. Mock-infected cells. f. HSV 1 infected cells not incubated with MA1147.

infection was inhibited. HSV 2 infection, on the other hand, was not inhibited by 5 mM neomycin, as illustrated in Fig.3 c and d. Uninfected cells (Fig.3 e) and infected cells not treated with antibody (Fig.3 f) were used as controls. The lack of effect of

neomycin on HSV 2 infection confirmed the results in Fig.1, where virus titre was counted.

Sodium sulfate concentrations corresponding to the concentrations of neomycin sulfate had no effect on virus infection (data not shown).

DISCUSSION

We have earlier shown that HSV 1 infection affects polyphosphoinositide metabolism in BHK cells (8). On this background we tested neomycin's effect on virus infection, since it has been shown to bind strongly to polyphoshoinositides both in vitro (9) and in vivo (2,3,10-13), thereby inhibiting phosphoinositide-mediated cellular events.

Surprisingly, the results show that neomycin concentrations above 3 mM almost completely abolished HSV 1 infection, while HSV 2 infection was unaffected. Therefore, the effect of neomycin seems to be highly specific. Since HSV 1 and HSV 2 have separate cellular receptors (6), this specificity can be explained by interference with virus-receptor interaction. This possibility was further supported by the time dependency of the neomycin effect (Fig.2). If the neomycin effect was at the (pre-)receptor level, it would have to be present before receptor binding/internalization of virus takes place. The time course given in Fig.2 corresponds well with the known time course of virus adsorption.

Plaque formation gives information about virus production. But the results given in Fig.3 indicated that also earlier stages of HSV 1 infection were inhibited. Preliminary data indicate that HSV 1 penetration into BHK cells is altogether abolished (not shown).

It remains to be investigated whether neomycin exerts its effect by affecting the virus particle or by altering the cellular receptor function. It is also possible that infection is stopped at a later, but still very early, stage of infection. Also, whether this highly specific inhibitory effect is really mediated through neomycin's

ability to bind strongly to polyphosphoinositides remains to be determined.

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