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Antiviral Effects of Hygromycin B, a Translation Inhibitor Nonpermeant to Uninfected Cells

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Hygromycin B, a preferential translation inhibitor in virus-infected cells, has been tested for its antiviral effects against herpes simplex virus type 2 and poliovirus. The activity has been compared with other antiviral agents such as vidarabine and iododeoxyuridine.

Permeability changes are induced by a number of animal viruses during infection (4, 8, 11). These modifications are observed during virus adsorption and late in the replicative cycle when most of the virion components are synthesized (3, 5, 9). Some inhibitors that do not usually cross the membrane of normal cells readily pass to virus-infected cells. Hygromycin B, gougerotin, blasticidin S, edeine, anthelmycin, and some nucleotide derivatives preferentially block translation in animal virus-infected cells as compared with their uninfected counterparts (2, 5, 10). The possibility that some of these inhibitors could behave as antiviral agents has already been suggested (2). In fact, hygromycin B blocks the production of vesicular stomatitis virus, herpes simplex virus type 1 (HSV-1), and Sendai virus at concentrations that do not block protein synthesis in uninfected cells (1). We used this inhibitor to test its activity in cell culture antiviral systems with HSV-2 and poliovirus as challenge viruses.

Confluent HeLa cells were grown in Linbro plates and infected or mock-infected at different multiplicities with poliovirus type 1 Mahoney strain or with HSV-2 in Dulbecco modified Eagle medium supplemented with 2% calf serum. Different concentrations of the compound were used as described in the legends to Fig. 1 and 2. After virus adsorption, i.e., 2 h of incubation at 37°C, the medium was replaced, and the appropriate concentrations of compound were added again. In the poliovirus HeLa cell system, the monolayers were analyzed under a phase-contrast Nikon microscope for cytopathic effect (CPE) at 30 h postinfection. Simultaneously, translation activity was measured in the presence of 0.5 μCi of [^{35}S]methionine per ml during incubation for 1 h at 37°C in Dulbecco modified Eagle medium without methionine. In HSV-2-infected HeLa cells, this analysis was carried out at 48 h postinfection.

We first analyzed the effect of hygromycin B at a concentration of 0.5 mM for the inhibition of PFU by a number of animal viruses. The inhibitory effect on cellular translation at this concentration was about 20% after 20 h of incubation. The degree of inhibition by hygromycin B differs with the different viruses used. The highest inhibition was observed in HSV-1 production (0.3% of control). Less inhibited were Newcastle disease virus and poliovirus (0.4 and 0.8% of control, respectively). The least inhibited virus was Semliki Forest virus (35% of control; data not shown). The differences in the inhibition of virus production by hygromycin B depends not only on the degree of membrane permeability but also on the effect that a partial inhibition of viral protein synthesis would have on the production of new infective particles.

Several systems developed to check the antiviral activity of new compounds are based on the protection of the cell monolayer from viral infection by using different concentrations of the compound to be tested (7). Figure 1 illustrates the CPE observed when HeLa cells were infected with HSV-2 at different multiplicities. The presence of hygromycin B at concentrations of 0.1, 0.2, and 0.5 mM prevented the cells from destruction by HSV-2 infection. These concentrations did not induce a CPE on control HeLa cells after 48 h of incubation. However, protein synthesis was totally blocked in the infected cells, and 75% inhibition of translation in uninfected cells occurred when 0.5 mM hygromycin B was present for 48 h. The effect of iododeoxyuridine and vidarabine in this system is shown in Fig. 1C, D, E, and F for comparison. With these

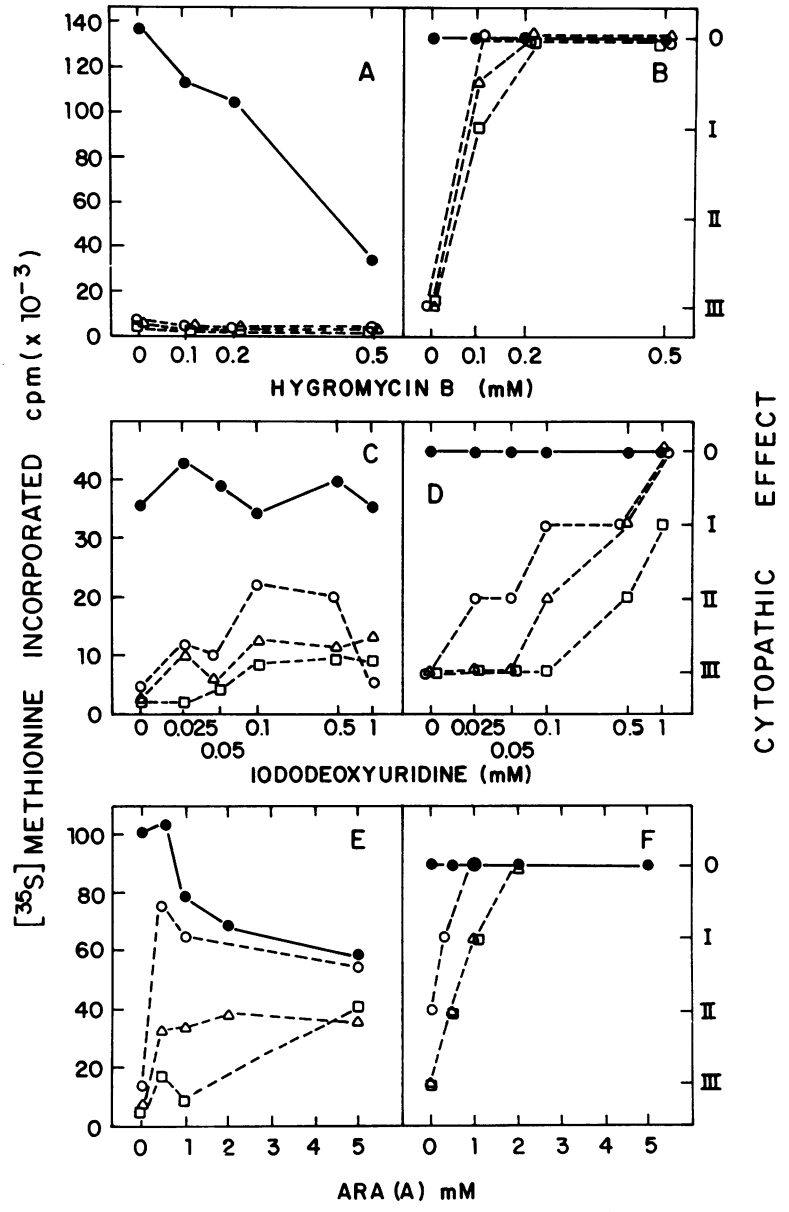

FIG. 1. Effect of compounds on HSV-2. HeLa cells were grown in 24-well Linbro dishes until confluence was reached. They were infected with HSV-2 at different multiplicities. ●, No HSV-2 added; ○, 0.4 PFU per cell; △, 2 PFU per cell; □, 4 PFU per cell. After 2 h of adsorption, the excess virus was removed and 1 ml of Dulbecco modified Eagle medium supplemented with 2% calf serum and containing the indicated concentration of compound was added. After 48 h of incubation at 37°C, the CPE was recorded under a phase-contrast Nikon microscope according to the following scale: 0, no CPE; I, about 25% cell destruction; II, about 50% cell destruction; III, more than 75% cell destruction. Then, protein synthesis was estimated by incubating the cells for 1 h in the presence of [^{35}S]methionine as already described (9). ARA(A), Vidarabine.

two compounds, a protective effect against the destruction of the cell monolayer by HSV-2 infection is apparent, and the concentrations needed to achieve this protection are comparable to those necessary for hygromycin B to achieve a similar effect. On the other hand, partial metabolic activity can be seen in cells infected with HSV-2 and treated with vidarabine or iododeoxyuridine, as shown by the level of protein synthesis in the cell monolayers after 48 h of infection.

Although a number of antiherpes drugs are

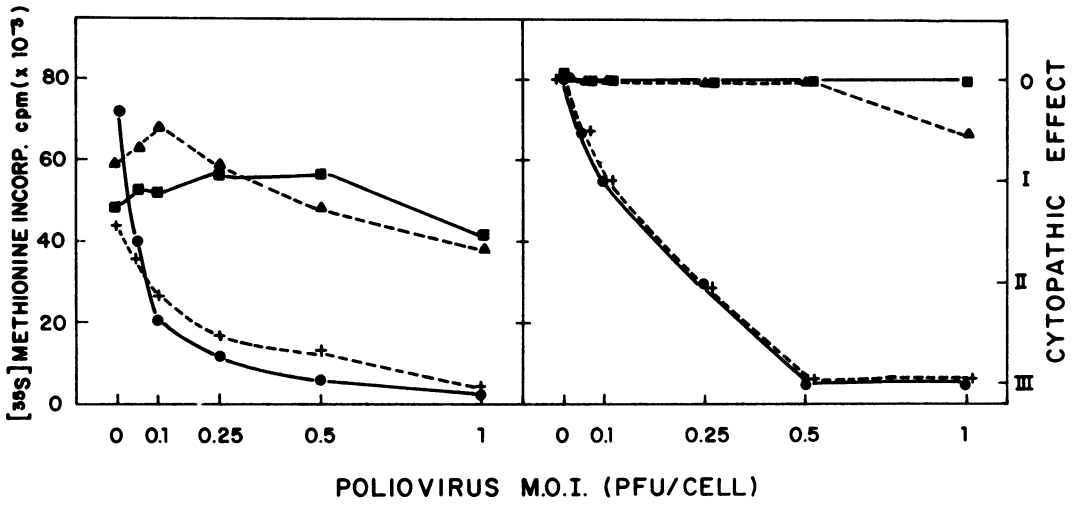

FIG. 2. Effect of hygromycin B on poliovirus. HeLa cells grown in 24-well Linbro dishes were infected with the indicated multiplicities of poliovirus. After 2 h of incubation, the medium was replaced by 1 ml of Dulbecco modified Eagle medium supplemented with 2% calf serum and containing 0.2 mM hygromycin B (▲), 0.5 mM hygromycin B (■), 10^{-7} M cycloheximide (+), or no compound (●). After 30 h of incubation at 37°C, the CPE and protein synthesis were measured as indicated in the legend to Fig. 1. M.O.I., Multiplicity of infection.

now available, it must be noted that the most potent compounds are all nucleoside derivatives, which are selectively recognized by viral enzymes. A mutation in the viral protein kinase renders HSV-1 resistant to several of these nucleoside derivatives (6). In addition, the risk of mutagenicity by these agents makes their use not advisable against relatively mild viral infections.

To test the effect of hygromycin B on a different virus, the experiment shown in Fig. 2 was carried out. HeLa cells were infected at different multiplicities of infection with poliovirus in the presence of 0.2 or 0.5 mM hygromycin B. As a control, we included the translation inhibitor cycloheximide at a concentration that induced about 30% inhibition of translation in normal cells. Figure 2 indicates that the CPE and protein synthesis are protected in cells infected with poliovirus and treated with hygromycin B, whereas cycloheximide has no protective effect. The concentration of cycloheximide used induced a partial blockade of protein synthesis in control cultures after 30 h of incubation which was similar to that observed under the highest concentration of hygromycin B. This result suggests that the protection conferred by hygromycin B on the cell monolayer is not a mere consequence of the partial inhibition of translation. Moreover, the finding that hygromycin B has antiviral activity in the two systems analyzed in this work favors the idea that these unpermeant compounds could be used as broad-spectrum antiviral agents. We would point out that, although hygromycin B is relatively toxic for use on humans, these results leave open the possibility of a search for antiviral compounds that show selected permeability to virus-infected cells.

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