Adamantanamine and Early Events Following Influenza Virus Infection

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With 2 Figures

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Summary

Treatment of chick embryo cells with 1-adamantanamine prevented the synthesis of fowl plague virus-directed RNA and the development of the virus-induced depression of cellular protein synthesis. The event sensitive to the compound preceded an early event sensitive to cycloheximide. Interferon production induced by ultraviolet-irradiated virus was depressed by the compound. These data suggest that 1-adamantanamine prevents the release of viral nucleic acid within the cell.

1. Introduction

The chemoprophylactic drug 1-adamantanamine (Symmetrel®) inhibits an early, unknown, event in the replication of some myxoviruses. Early reports (1, 2, 3) indicated that the compound did not block virus adsorption, but did inhibit penetration of virus into cells (1, 2). Penetration was measured as a loss of virus neutralizability by antiserum added to the virus-cell complex. Later, Kato and Eggers (4), who could not confirm that penetration was inhibited, showed that uncoating of the virus, defined as a loss of photosensitivity of neutral red-labelled virus in infected cells, was depressed by the compound. These data are difficult to interpret, particularly because the temporal and spatial relationships between myxovirus adsorption, penetration and uncoating are not clear (5). It was therefore of interest to investigate the effect of the compound on other early events following infection. The present report confirms that 1-adamantanamine acts at some stage between virus adsorption and the release of viral nucleic acid within the cell.

2. Materials and Methods

1-adamantanamine was a gift from Dr. A. W. Galbraith, Pharmaceuticals Division, Geigy (U.K.) Ltd. Cells, media and virus, and measurement of the rate of protein
synthesis in infected cells were as previously described (6). Interferon production and assay, and incorporation of radioactive uridine into viral RNA, extraction of RNA from infected cells and fractionation of RNA on polyacrylamide gel were as described by Gandhi and Burke (7).

3. Results

Preliminary experiments indicated that 25 μg/ml of 1-adamantanamine inhibits the replication of fowl plague virus (FPV; avian influenza A) in chick embryo cells without affecting the adsorption of the virus.

Among the first events which occur when the nucleic acid of the infecting virus is exposed within the cell is the initiation of virus-directed RNA synthesis. Fowl plague virus RNA synthesis can readily be detected when cell RNA synthesis is depressed by addition of actinomycin late in the replicative cycle. Earlier addition of the compound inhibits an unknown event necessary for virus replication. Figure 1 shows the resolution on polyacrylamide gel of several species of

Fig. 1. Detection of FPV-directed RNA synthesis
Polyacrylamide gel electrophoresis of RNA extracted from (A) uninfected cells receiving actinomycin 1 hour before addition of [3H]-uridine for a further 2 hours (B) FPV-infected cells receiving actinomycin at 4 hours after infection and [3H]-uridine between 4½ and 6½ hours after infection. Arrows indicate the positions of co-electrophoresed [14C]-labelled ribosomal RNA. Migration was from left to right. Further details are as described by Gandhi and Burke (7).

3H-uridine-labelled RNA extracted from infected actinomycin-receiving cells, which were not synthesised in similarly treated uninfected cells. A similar separation of FPV RNA in infected cells was obtained by Gandhi and Burke (7). These viral RNA species could not be detected when 1-adamantanamine was added 1 hour before infection (Fig. 2B) suggesting that the compound prevented the synthesis of viral RNA. A control experiment (Fig. 2A) shows that 1-adamantanamine did not affect cell RNA synthesis.