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Monensin and Nigericin Prevent the Inhibition of Host Translation by Poliovirus, without Affecting p220 Cleavage

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Addition of monensin or nigericin after poliovirus entry into HeLa cells prevents the inhibition of host protein synthesis by poliovirus. The infected cells continue to synthesize cellular proteins at control levels for at least 8 h after infection in the presence of the ionophore. Cleavage of p220 (γ subunit of eukaryotic initiation factor 4 [eIF-4γ]), a component of the translation initiation factor eIF-4F, occurs to the same extent in poliovirus-infected cells whether or not they are treated with monensin. Two hours after infection there is no detectable intact p220, but the cells continue to translate cellular mRNAs for several hours at levels similar to those in uninfected cells. Nigericin or monensin prevented the arrest of host translation at all the multiplicities of poliovirus infection tested. At high multiplicities of infection, an unprecedented situation was found: cells synthesized poliovirus and cellular proteins simultaneously. Superinfection of vesicular stomatitis virus-infected HeLa cells with poliovirus led to a profound inhibition of vesicular stomatitis virus protein synthesis, while nigericin partially prevented this blockade. Drastic inhibition of translation also took place in influenza virus-infected Vero cells treated with nigericin and infected with poliovirus. These findings suggest that the translation of newly synthesized mRNAs is dependent on the integrity of p220, while ongoing cellular protein synthesis does not require an intact p220. The target of ionophore action during the poliovirus life cycle was also investigated. Addition of nigericin at any time postinfection profoundly blocked the synthesis of virus RNA, whereas viral protein synthesis was not affected if nigericin was added at 4 h postinfection. These results agree well with previous findings indicating that inhibitors of phospholipid synthesis or vesicular traffic interfere with poliovirus genome replication. Therefore, the action of nigericin on the vesicular system may affect poliovirus RNA synthesis. In conclusion, monensin and nigericin are potent inhibitors of poliovirus genome replication that prevent the shutoff of host translation by poliovirus while still permitting cleavage of p220.

Picornaviruses cause a profound inhibition of cellular protein synthesis during infection (6, 12, 30). Two different mechanisms have been invoked to account for this blockade. In the case of cardioviruses, such as encephalomyocarditis virus, alterations of intracellular ion concentrations are probably responsible for the arrest of cellular protein synthesis late during infection (7, 9). These ionic alterations are the consequence of membrane leakiness resulting from the synthesis of viral macromolecules, but the exact viral protein responsible for increasing membrane permeability remains to be identified (8). The finding that the picornavirus mRNA lacks a cap structure at the 5′ end led to the suggestion that selective inactivation of an initiation factor(s) involved in cap recognition could be responsible for the inhibition of host translation during picornavirus infection (for reviews, see references 6, 12, and 30). The discovery that a p220 polypeptide involved in cap recognition was proteolytically cleaved during poliovirus infection provided a first explanation for this puzzling phenomenon (14). This finding suggested that p220 was a key component of a new initiation factor, subunit F of eukaryotic initiation factor 4 (eIF-4F). In fact, the main evidence that p220 could participate in translation came from the finding that p220 was cleaved during poliovirus infection (14). Later studies indicated that there is no absolute correlation between the degree of p220 cleavage and the severity of the reduction of host translation (5, 22, 27). Infection of HeLa cells with poliovirus in the presence of inhibitors of viral genome replication allowed almost complete cleavage of p220, while the cells still synthesized substantial amounts (50 to 80%) of host proteins (5, 27). On the other hand, poliovirus-infected cells transferred to 28°C after infection undergo proteolytic degradation of p220, but cellular translation continues, even if the cells are shifted up to 37°C (27). The combination of a low temperature (28°C) and guanidine indicated that cellular protein synthesis may continue unabated for 1 to 2 h in poliovirus-infected cells in which no intact p220 is detected (27). Finally, a human erythroleukemic cell line can be persistently infected with poliovirus, under conditions in which substantial degradation of p220 occurs, without host translation being affected at all (22). Taken together, these findings suggested that cleavage of p220 does not necessarily lead to the blockade of ongoing cellular translation.

The use of inhibitors of poliovirus genome replication demonstrated that the translation of input poliovirus RNA is sufficient to interfere with cellular translation (10). To date, no inhibitors of the shutoff of host protein synthesis induced by poliovirus have been found under conditions in which viral proteins are synthesized to the same levels as in control untreated cells. Such inhibitors would provide a unique tool with which to obtain insights into the molecular basis of the blockade of host translation by picornaviruses. We now report that the ionophores monensin and nigericin potently prevent the arrest of host translation by poliovirus. These inhibitors behave in a way similar to that previously reported for guanidine, 3-methylquercetin (5), or brefeldin A (18), but they block the shutoff of host translation even more potently than the previously described compounds. Evidence that the mode of action of nigericin against poliovirus lies in its ability to interfere with viral RNA synthesis is also provided. This inhibition is likely

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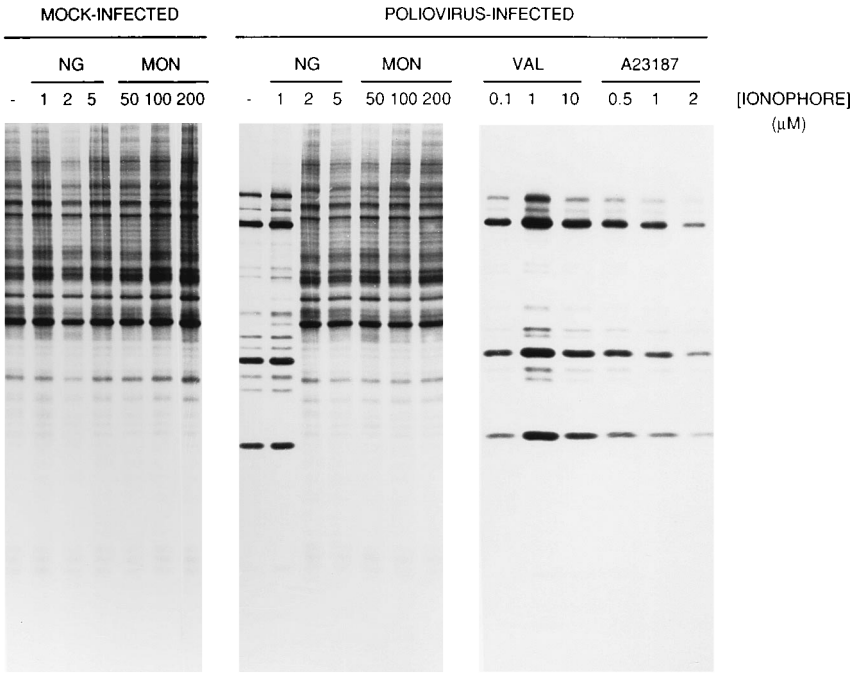

FIG. 1. Effects of several ionophores on protein synthesis in HeLa cells infected with poliovirus. HeLa cells were mock infected or infected with poliovirus (10 PFU per cell). After adsorption for 1 h, new medium containing different concentrations of nigericin (NG), monensin (MON), valinomycin (VAL), or A23187 was added to both mock-infected and poliovirus-infected HeLa cell monolayers. Proteins were labeled at 5 hpi and analyzed as described in Materials and Methods. Lanes −, no compound added.

mediated by the effects of these ionophores on the vesicular system.

MATERIALS AND METHODS

Cell cultures and virus. Dulbecco modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum was used for growth and maintenance of HeLa or Vero cell cultures. Poliovirus type 1 (Mahoney strain) or vesicular stomatitis virus (VSV) was propagated, grown, and titrated by plaque assay in HeLa cells. Influenza virus (Victoria strain) was grown and titrated on MDCK cells. The viral infections were performed in DMEM with 2% newborn calf serum (poliovirus and VSV) or without serum (influenza virus). The multiplicities of infection (MOI) are indicated below.

Antibiotics. Nigericin, A23187, valinomycin, and monensin were purchased from Sigma. Actinomycin D was provided by Boehringer Mannheim.

Analysis of proteins by PAGE and autoradiography. HeLa cell monolayers were infected as described above, in DMEM containing 2% calf serum. Virus adsorption was allowed to continue for 30 min at 37°C. Proteins were labeled by the addition of 20 μCi of [^{35}S]methionine per ml (1,220 Ci/mmol; Amersham) contained in methionine-free DMEM. After 1 h of incubation with the radioactive medium, the label was removed and the cells were harvested in 0.1 ml of sample buffer (17). The samples were sonicated at 12 Hz and boiled for 5 min, and then 15 μl of the samples was electrophoresed overnight at 80 V by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fluorography and autoradiography of the gels were done as described previously (18). To estimate total radioactivity incorporated, 10-μl aliquots were trichloroacetic acid precipitated and filtered through glass fiber filters as described elsewhere (10).

Immunoblot analysis. A 50-μl volume of the [^{35}S]methionine-labeled sample was applied and run in an SDS–7.5% polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane (Trans-blot transfer medium; Bio-Rad) overnight at 200 mA in a transfer buffer (25 mM Tris-HCl [pH 8.3], 90 mM glycine, 20% methanol, and 0.2% SDS). The nitrocellulose sheet was stained with Ponceau S (Sigma). A 5% (wt/vol) solution of nonfat dry milk in phosphate-buffered saline (PBS) was used to block the membrane by rocking for 1 h at room temperature. The mixture of anti-p220 polyclonal antibodies was diluted (1:1,000) in a 1% dry milk solution, and the blot was incubated with the anti-p220 antibodies for 2 h and then washed with four changes of PBS containing 0.05% Tween 20 (Sigma). A second incubation with biotinylated goat anti-rabbit antibody (1:10,000) (Vector Laboratories) was carried out for 1 h before four more washes. The blot was incubated with streptavidin-peroxidase conjugate (Boehringer Mannheim) for 30 min. Conjugates were detected by using a luminol-luciferin system (28, 34). Finally, the blot was allowed to dry before exposure to an X-ray film (Agfa) for about 2 min at room temperature.

Incorporation of ^{3}H-labeled uridine in RNA. Monolayers of 5×10^5 HeLa cells were infected with poliovirus at an MOI of 50 in the presence of 5 μg of actinomycin D per ml. [5′-^{3}H]uridine (10 μCi/ml) was added 1 h before the times indicated in the legend to Fig. 6. After a 60-min labeling period, the medium containing the label was discarded, and the cells were treated with 0.5 ml of 5% trichloracetic acid, washed twice with ethanol, dried under an infrared lamp, and dissolved in 200 μl of 0.1 N NaOH–1% SDS. The radioactivity in 150-μl samples was determined with a liquid scintillation spectrometer.

RESULTS

Nigericin prevents the poliovirus-induced shutoff of host translation. During the course of previous studies of inhibitors of the vesicular system that interfere with the replication of animal virus genomes (17, 18), nigericin was tested against poliovirus. Surprisingly, it was found that concentrations of this carboxylic ionophore as low as 2 μM completely prevented the inhibition of host translation by poliovirus and also blocked the synthesis of viral proteins (Fig. 1). This phenomenon was observed when the ionophore was added after adsorption of poliovirus for 1 h, when the virus inoculum had been removed. Concentrations of nigericin or monensin of 5 and 200 μM, respectively, had no adverse effects on HeLa cells, even after 5 h of incubation, as observed by phase-contrast microscopy or by measurement of protein synthesis (Fig. 1).

To test if other ionophore molecules had effects similar to that of nigericin, the action of monensin, valinomycin, and A23187 was analyzed. Monensin, which is also a carboxylic ionophore, had an effect similar to that of nigericin, whereas valinomycin (a potassium transporter) or A23187 (a calcium ionophore) did not prevent poliovirus-induced shutoff of cellular protein synthesis. Thus, the ionophores nigericin, which exchanges K^+ for H^+, and monensin, which exchanges Na^+ for H^+, block the synthesis of poliovirus proteins, while preventing virus-mediated inhibition of cellular translation. These two carboxylic ionophores are known to interfere with the vesicular system (25, 32).

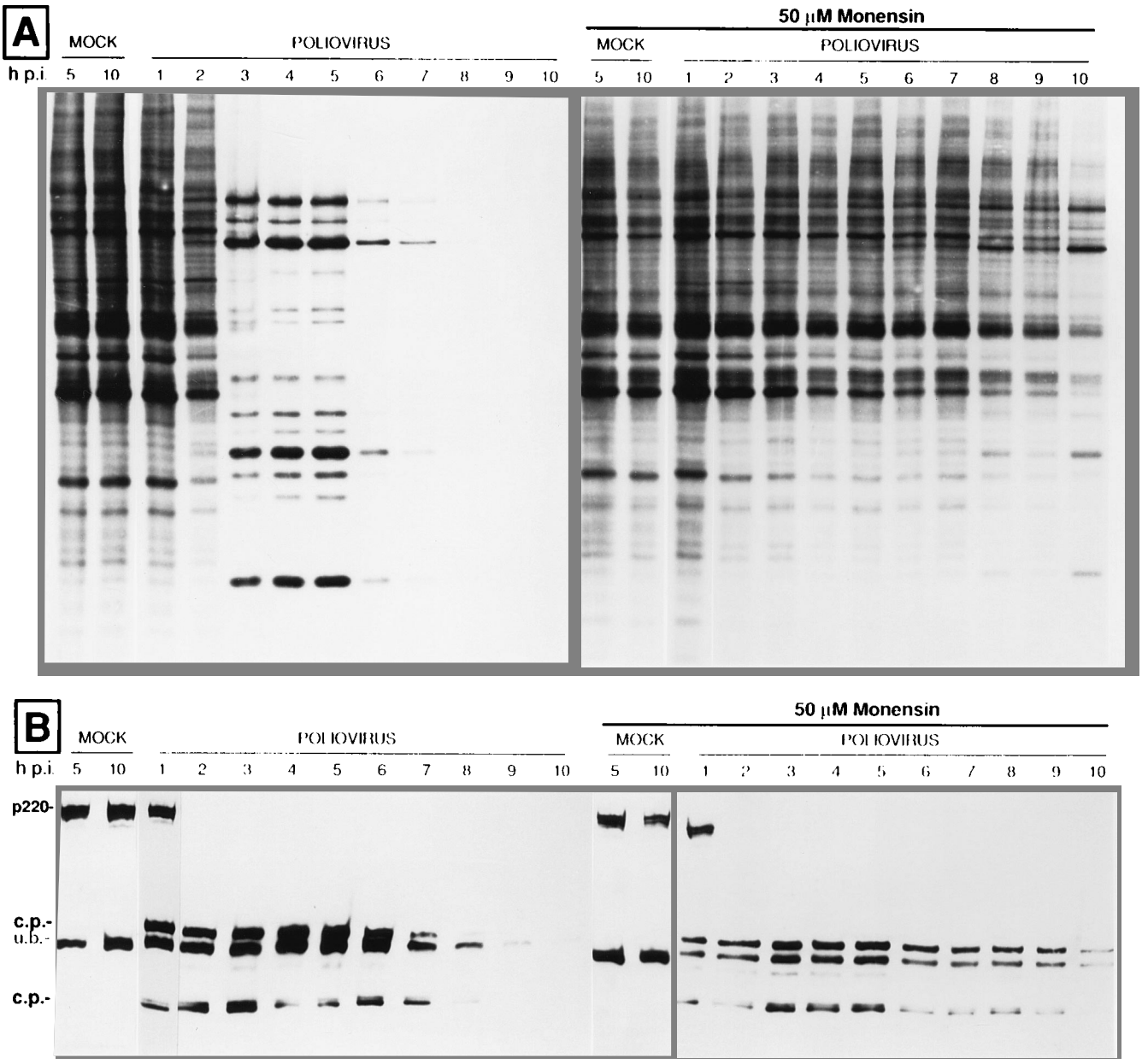

FIG. 2. Effects of monensin on the kinetics of poliovirus protein synthesis and p220 cleavage in HeLa cells. (A) Protein synthesis. Monensin (50 μM) was added after 1 h of viral adsorption to both mock-infected and poliovirus-infected HeLa cells (MOI, 10). At the indicated times postinfection, the medium containing monensin was removed and proteins were labeled for 1 h by addition of radioactive methionine and analyzed by SDS–15% PAGE. (B) Analysis of p220 cleavage. The same samples were applied to an SDS–7.5% polyacrylamide gel, transferred to nitrocellulose paper, and incubated with p220 polyclonal antibodies as described in Materials and Methods. c.p., p220 cleavage products; u.b., unspecific band.

Monensin and nigericin do not interfere with the cleavage of p220 (eIF-4γ). Since monensin and nigericin potently blocked the shutoff of host translation by poliovirus when added after virus entry, we analyzed the kinetics of protein synthesis and the cleavage of p220 (eIF-4γ) in parallel in poliovirus-infected cells which were untreated or treated with monensin. Figure 2A shows the kinetics of poliovirus infection in HeLa cells grown in monolayers. The replication of poliovirus in these cells is slower than that in suspension cultures, and poliovirus protein synthesis is detected only after 3 h postinfection (3 hpi). Poliovirus-infected cells clearly synthesized cellular proteins even after 9 hpi, when monensin was continuously present after virus entry. The synthesis of some poliovirus proteins is apparent in monensin-treated cells after 6 to 7 hpi, suggesting that the blocking of poliovirus replication by monensin is not complete, at least under these conditions.

The analysis of p220 cleavage shows little difference between control cells infected by poliovirus and those treated with 50 μM monensin (Fig. 2B). Cleavage of p220 can be detected as early as 1 hpi. Two to three hours after infection, no intact p220 is detected in poliovirus-infected cells in either the absence or the presence of monensin. Despite this extensive proteolytic cleavage of p220, cellular protein synthesis continues for several hours in the presence of the ionophore. These findings, therefore, provide additional evidence that the translation of cellular mRNAs does not require the presence of an

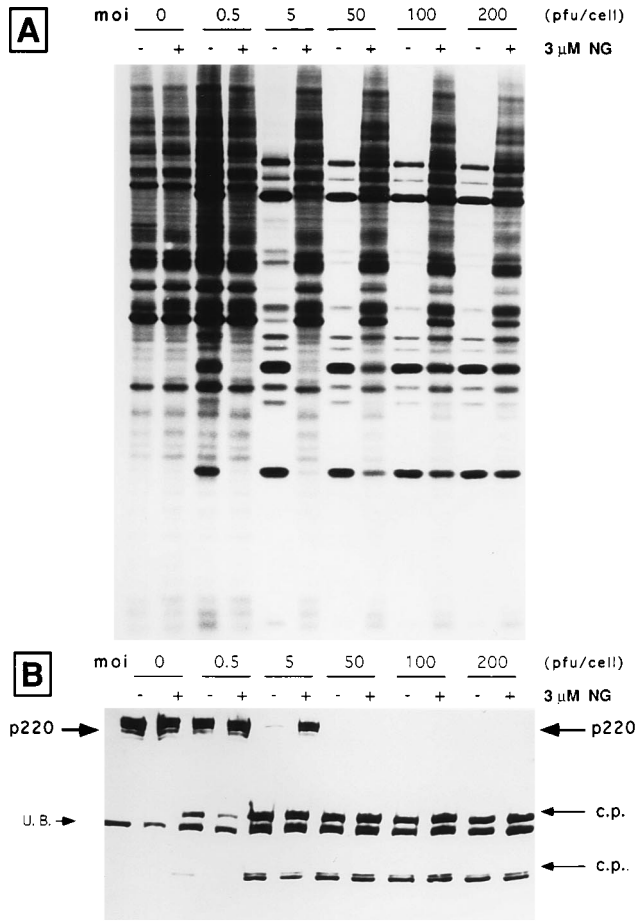

FIG. 3. Influence of the multiplicity of poliovirus infection on the effect of nigericin. (A) Protein synthesis; (B) analysis of p220 cleavage. HeLa cells were mock infected or infected with poliovirus at the indicated MOIs. After viral adsorption, nigericin (NG) was added where indicated (lanes +). Proteins were labeled at 5 hpi. Cells were harvested 1 h later and processed as described in Materials and Methods and in the legend to Fig. 2. c.p., p220 cleavage products; U.B., unspecific band.

intact p220. Moreover, it appears that monensin treatment does not hamper the translation of the input poliovirus RNA because $2A^{pro}$ is made, as judged by p220 cleavage. Essentially similar results are found when 3 μM nigericin is used, i.e., no shutoff of host translation by poliovirus takes place, while p220 becomes cleaved. Although no inhibition of protein synthesis is found when cells are treated with 50 μM monensin or 3 μM nigericin, we have found that monensin is less toxic to cells than nigericin. Nevertheless, the findings described in this article are equally applicable to monensin and nigericin.

The effects of nigericin on poliovirus translation and p220 cleavage at different MOIs are shown in Fig. 3. The presence of this ionophore allowed the synthesis of cellular proteins even at a high MOI. Even more striking was the finding that at high MOIs (50 PFU per cell or greater) poliovirus protein synthesis is clearly seen and coexists with the translation of cellular mRNAs. This is, to our knowledge, an unprecedented phenomenon in poliovirus-infected HeLa cells. The integrity of p220 was examined in parallel (Fig. 3B). Virtually all p220 is degraded at high MOIs. In this experiment, some uncleaved p220 was present in cells infected at 5 PFU per cell and treated with nigericin. Nevertheless, nigericin interferes with the poliovirus-induced arrest of host translation, even when high MOIs are employed and poliovirus proteins are synthesized, leading to complete cleavage of p220.

Effects of nigericin on translation in doubly infected cells. The results described above indicated that p220 integrity was not necessary for reinitiation of mRNAs already engaged in translation. Our interest was in assaying the effect of p220 cleavage on the translation of newly synthesized mRNAs containing a cap structure at their 5′ ends. To this end, double infection with poliovirus and other animal viruses was assayed. Superinfection with poliovirus of human cells previously infected with VSV leads to a profound inhibition of VSV mRNA translation (2, 13). The molecular basis of this effect has not been thoroughly investigated, but it is thought that cleavage of p220 by poliovirus infection would block protein synthesis on capped mRNAs, such as those of VSV. To test the action of nigericin on translation in HeLa cells doubly infected with poliovirus and VSV, cells were simultaneously infected with the two viruses and the ionophore was added later, after removal of the virus inocula. However, nigericin interfered with VSV replication (results not shown), in agreement with the idea that genome replication is dependent on an intact vesicular system not only in poliovirus-infected cells, but also in VSV-infected cells (19). Therefore, the protocol shown in Fig. 4A was followed. Cells were initially infected with VSV to allow its replication and the appearance of significant levels of VSV mRNA translation (Fig. 4C). Addition of 3 μM nigericin after 4.5 h of incubation of the VSV inoculum still allows the synthesis of VSV proteins for several hours after nigericin addition. Moreover, nigericin shows a clear reduction of the shutoff of host translation induced by VSV (Fig. 4B and C). This effect is particularly striking at 4 to 5 h after nigericin addition (Fig. 4C). When VSV-infected cells are superinfected with poliovirus in the absence of the ionophore, there is a dramatic decrease of VSV protein synthesis, while poliovirus proteins become clearly apparent, in agreement with previous findings (2, 13). Moreover, p220 cleavage occurs in these doubly infected cells, similar to the results for control cells infected with only poliovirus (Fig. 4C). Notably, when the doubly infected HeLa cells are treated with nigericin, poliovirus protein synthesis is suppressed, but not p220 cleavage, while substantial amounts of VSV proteins are still synthesized, particularly for 2 to 3 h after poliovirus and nigericin addition. However, a clear reduction of VSV translation is observed after 3 h (Fig. 4C). The quantitation of total radioactivity incorporated at the various times and under the different conditions used is shown in Fig. 4B. It seems clear that nigericin partially prevents the inhibition of translation both in VSV-infected cells and in doubly infected cells. We interpret these results to mean that ongoing translation of VSV mRNAs is not dependent on the integrity of p220, while the newly synthesized VSV mRNAs may require an intact p220 to be translated by the protein-synthesizing machinery. Alternatively, it is also possible that the cleaved p220 retains some activity which allows it to participate in the reinitiation of translation, at least for a few hours.

The influenza virus mRNAs not only contain a cap structure at their 5′ ends, but even the initial nucleotides of viral mRNAs are stolen from cellular mRNAs during viral transcription (20). Contrary to the case for VSV, which synthesizes its mRNAs in the cytoplasm, influenza virus transcription occurs in the nucleus of the infected cells, and the mRNAs synthesized need to be transported to the cytoplasm to be translated (20). Thus, it was of interest to assay the effects of p220 cleavage on influenza virus mRNA translation, under conditions in which poliovirus replication was restricted by nigericin. To this end, we chose the Victoria strain of influenza virus for infection of Vero cells, which are also fully permissive for poliovirus. Ad-

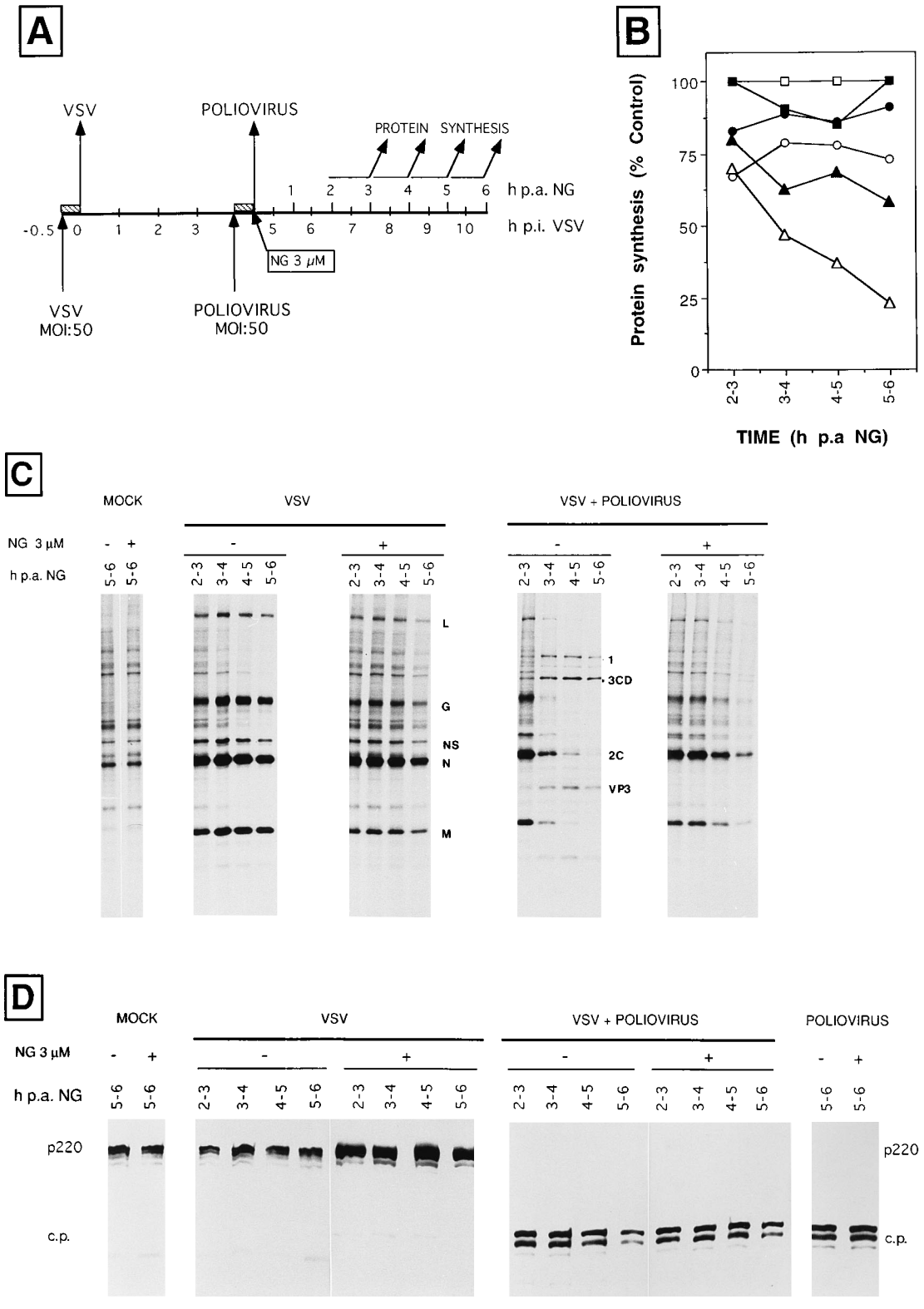

FIG. 4. Effect of nigericin on translation in HeLa cells doubly infected with poliovirus and VSV. (A) Schematic representation of the protocol followed in the experiment. (B) Cell monolayers were labeled as described in the legend to Fig. 1 and dissolved in 100 μl of sample buffer. A 10-μl aliquot of this sample was precipitated with trichloroacetic acid and filtered through glass fiber filters. The radioactivity retained in the filters was measured with a liquid scintillation counter. □, control HeLa cells; ○, VSV-infected HeLa cells; △, poliovirus-infected HeLa cells; ■, control HeLa cells plus 3 μM nigericin; ●, VSV-infected HeLa cells plus 3 μM nigericin; ▲, HeLa cells doubly infected with VSV and poliovirus plus 3 μM nigericin. (C) The proteins synthesized at the times indicated were separated on SDS-polyacrylamide gels and analyzed as described in Materials and Methods. The VSV proteins (L, G, NS, N, and M) and some poliovirus proteins (1, 3CD, 2C, and VP3) are indicated. (D) Analysis of p220 cleavage was carried out in parallel as described in Materials and Methods. c.p., cleavage products; h p.a. NG, hours post addition of nigericin.

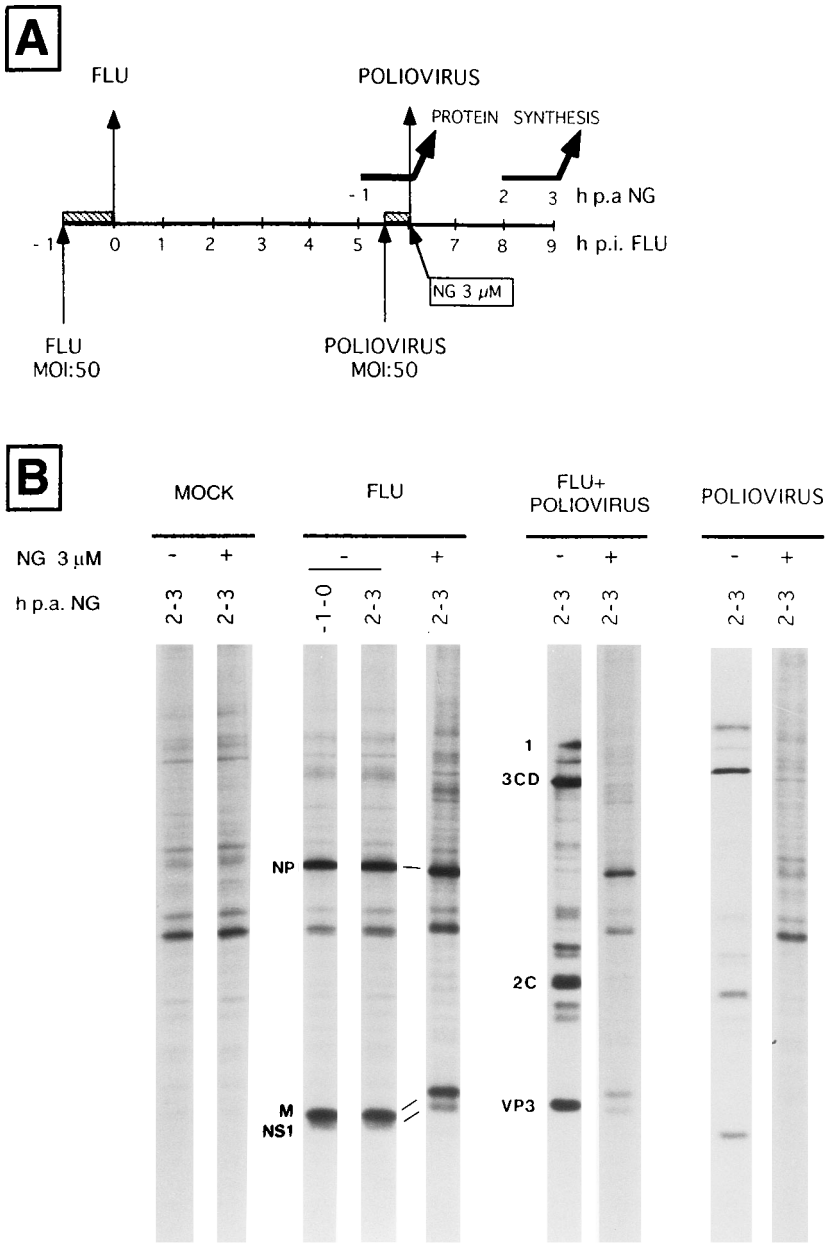

FIG. 5. Effects of nigericin on translation in Vero cells doubly infected with poliovirus and influenza virus. (A) Schematic representation of the protocol followed in the experiment. (B) Cell monolayers were labeled as described in the legend to Fig. 1 and dissolved in 100 μl of sample buffer. A 10-μl aliquot of this sample was applied to an SDS-polyacrylamide gel, and the labeled proteins were analyzed as described in Materials and Methods. The influenza virus proteins (NP, M, and NS1) and the poliovirus proteins (1, 3CD, 2C, and VP3) are indicated. (The influenza virus bands do not line up in all the lanes because samples were run in two independent gels.) FLU, influenza virus; h p.a. NG, hours post addition of nigericin.

dition of nigericin 5.5 h after infection of Vero cells with influenza virus has no effect on translation of influenza virus mRNAs (Fig. 5A), while the ionophore prevents poliovirus replication. Superinfection of influenza virus-infected cells with poliovirus leads to a drastic blockade of influenza virus protein synthesis, and almost exclusive translation of poliovirus mRNA takes place in these doubly infected cells. By contrast, treatment with nigericin abolishes poliovirus translation as expected, but the synthesis of influenza virus proteins is greatly compromised also under these conditions. Densitometric quantitation indicates that there is 75% (NP protein) to 90% (M plus NS1 proteins) inhibition of influenza virus protein translation in cells treated with nigericin and infected with poliovirus compared with that of nigericin-treated cells infected with influenza virus (Fig. 5). These results suggest that although some translation of influenza virus mRNAs is still observed when p220 is cleaved, there is a profound inhibition of influenza virus protein translation in the absence of an intact p220.

Nigericin is a potent inhibitor of the synthesis of poliovirus RNA. Previous work in our laboratory indicated that the replication of poliovirus genomes required continuous phospholipid synthesis (17). Moreover, compounds that interfere with the traffic of membranes in the vesicular system prevent the synthesis of poliovirus RNA (18, 23). Since nigericin and monensin are known to affect the intracellular vesicular traffic, the

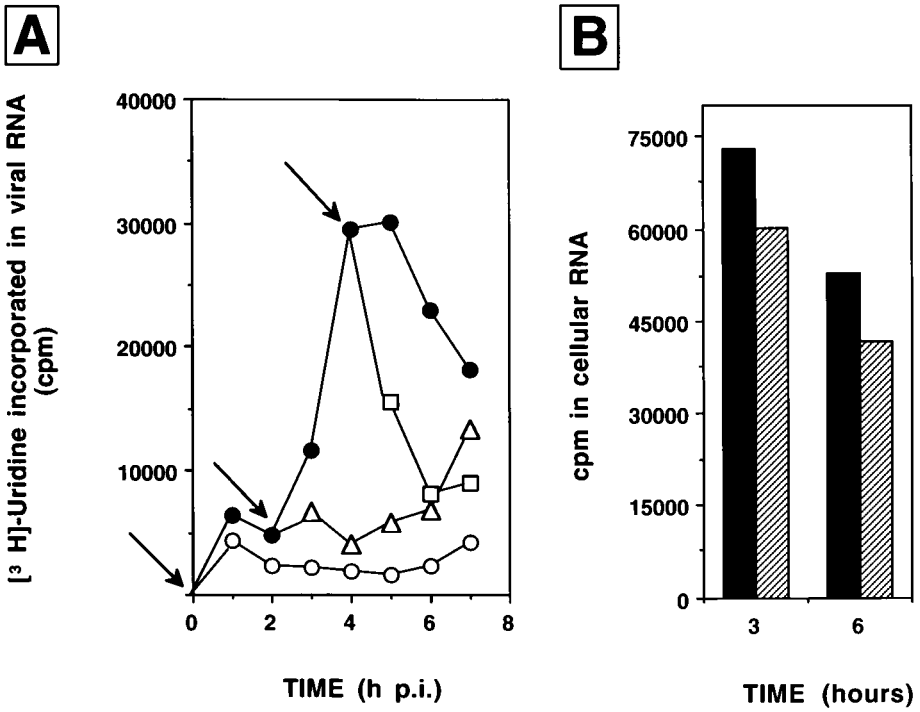

FIG. 6. Effect of nigericin on RNA synthesis in poliovirus-infected cells and in mock-infected HeLa cells. (A) Nigericin (3 μM) was added at 0 (open circles), 2 (open triangles), or 4 (open squares) hpi with poliovirus (10 PFU per cell) (arrows), and viral RNA was labeled at each hour postinfection. (B) RNAs from mock-infected cells treated with 3 μM nigericin (striped bars) for the indicated times or untreated (black bars) were also labeled.

action of nigericin on poliovirus RNA synthesis was assayed. Clearly, addition of nigericin at any time during poliovirus infection lowers the level of incorporation of uridine into viral RNA (Fig. 6). This effect is similar to the action of brefeldin A or other compounds that interfere with poliovirus genome replication (16, 18). A similar protocol to analyze the action of nigericin on viral protein synthesis shows that the ionophore allows the synthesis of poliovirus proteins when added late during infection (results not shown), suggesting that viral translation as such is not the target of nigericin. Finally, nigericin inhibits cellular RNA synthesis only slightly (Fig. 6B). Our conclusion from these results is that nigericin potently blocks poliovirus RNA synthesis, without affecting viral translation per se, as occurs with compounds that block the vesicular system (18, 23).

DISCUSSION

The mechanism by which poliovirus interferes with the translation of cellular mRNA is still a subject of intense debate (6, 8, 29, 30). The difficulty in resolving this question arises mainly from two problems: (i) there are no known poliovirus mutants defective in the shutoff of host translation and (ii) the putative poliovirus protein involved in the shutoff phenomenon has not been expressed individually in mammalian cells and shown to block ongoing cellular translation. In regard to the first point, some poliovirus mutants with alterations in the protease $2A^{pro}$ which were unable to cleave p220 were described previously (4). These mutants inhibited cellular translation with kinetics similar to those of wild-type poliovirus, but they had defects in the synthesis of late poliovirus proteins. Thus, it was concluded that an unspecific blockage of both viral and cellular translation occurred (4). This inhibition of translation was ascribed to the phosphorylation of eIF-2α (26) or to modifications in membrane permeability (15). We now know that $2A^{pro}$ is required for replication of poliovirus genomes (35). Most probably, the $2A^{pro}$ mutants unable to cleave p220 had defects in viral RNA synthesis (24). Therefore, the situation observed with the $2A^{pro}$ mutants described previously (4) was similar to that found with inhibitors of viral genome replication, i.e., inhibition of cellular translation still occurs, but late viral proteins are not synthesized (10, 16). In fact, these $2A^{pro}$ mutants served to illustrate that the arrest of cellular mRNA translation takes place efficiently even when p220 remains intact (4). The isolation of poliovirus mutants that do not inhibit host protein synthesis is still awaited (35).

Our present findings illustrating that monensin and nigericin prevent the shutoff of host translation by poliovirus can circumvent the difficulty in isolating these poliovirus mutants. These ionophores prevent poliovirus-induced shutoff of translation, while still permitting the translation of the input viral RNA. The ionophore must be present continuously to show this effect, since removal of nigericin at any time triggers poliovirus RNA replication after several hours (unpublished observation). This represents a further advantage of these inhibitors, because their reversible action makes them amenable to analysis of the mechanism of the inhibition of host translation by poliovirus. We believe that the present findings provide compelling evidence that ongoing cellular protein synthesis is not halted by cleavage of p220 (5, 22, 27).

Another important question that the present findings raise is the actual function of p220. The important discovery that poliovirus infection caused cleavage of p220 (14) focused attention on the role of this protein in the translation process (30). Since p220 forms part of eIF-4F, an initiation factor involved in cap recognition, it was thought that p220 was selectively required to translate only capped mRNAs, while naturally uncapped mRNAs, such as picornavirus RNA, would circumvent

this cap recognition step. However, addition of this protein to cell-free systems stimulates the translation of both capped and naturally uncapped mRNAs (3, 33). These findings contrast with the results obtained from mixed-infection experiments in which poliovirus or encephalomyocarditis virus RNA translation occurs efficiently in cells in which p220 has been proteolytically cleaved (1). Few studies of the effects of depleting p220 in cell-free systems by addition of purified picornavirus $2A^{pro}$ are available. Under these conditions, significant but not total inhibition of globin mRNA translation occurred, while translation of mRNA containing the picornavirus leader sequence was enhanced (21). The lack of in vivo experiments to assay the effects of p220 cleavage on the translation of cellular mRNAs by the individual expression of poliovirus $2A^{pro}$ makes it difficult at present to define a function for p220. Nevertheless, attempts to examine the effects of $2A^{pro}$ on the expression of a reporter gene (but not on ongoing cellular protein synthesis) show that the integrity of p220 is necessary for at least some steps of gene expression (11, 31). Measurement of the action of poliovirus $2A^{pro}$ on plasmid replication or transcription or translation of the chloramphenicol acetyltransferase gene, used as a reporter gene, suggested that the primary effect of $2A^{pro}$ action occurred at the transcriptional level (11). Our present findings provide a clear example of a situation in which no intact p220 is detected but cellular translation is still not inhibited. Certainly, under the conditions described here, there must be reinitiation of cellular mRNA translation, suggesting that intact p220 is not required to reinitiate the translation of the cellular mRNAs already engaged in translation. The system described here is well suited to testing of the consequences of p220 cleavage on the initiation of an incoming mRNA, either from the nucleus or transfected in the cytoplasm. Finally, our findings that VSV and, even more, influenza virus protein synthesis is severely compromised in Vero cells infected with poliovirus in the presence of nigericin suggests that p220 may be necessary for the translation of newly synthesized viral mRNAs. For influenza virus, these mRNAs need to be transported from the nucleus to the cytoplasm to be recognized by the protein-synthesizing machinery (20). Additional studies of the system described here would shed more light on the exact participation of p220 in gene expression.

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