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The antiviral compound 5-(3,4-dichlorophenyl) methylhydantoin inhibits the post-synthetic cleavages and the assembly of poliovirus in a cell-free system

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Abstract

The mode of action of the antiviral drug 5-(3,4-dichlorophenyl) methylhydantoin (hydantoin) was studied in a cell-free system allowing de novo synthesis of poliovirus. This cell-free system, which is programmed with viral RNA, is suitable for the study of the late stages of poliovirus replication and, thus, for a study of antiviral compounds acting on these late stages. It was shown that, apart from the known inhibition of the assembly of poliovirus, hydantoin also blocks post-synthetic cleavages of poliovirus proteins. Our data demonstrate that the cell-free system is a sensitive tool to study the mode of action of antiviral compounds.

Keywords: Poliovirus; 5-(3,4-dichlorophenyl) methylhydantoin; Antiviral drug; Cell-free system

1. Introduction

The Picornaviridae family is one of the most thoroughly investigated virus families. The picornaviruses possess a positive-stranded RNA genome, packaged in an icosahedrally symmetric protein capsid, composed of 60 copies each of VP1, VP2, VP3 and VP4 (Rueckert, 1996).

As several economically important viruses belong to the Picornaviridae (Hepatitis A virus, the human rhinoviruses, foot-and-mouth disease virus), the search for anti-picornavirus compounds to combat the diseases caused by picornaviruses has a long history. Although clinically approved therapeutic agents have not yet been developed, several picornavirus inhibitors have proven to be useful in studies of picornavirus molecular biology. A large number of compounds are available for the selective blockade of the different steps of the picornavirus replication cycle. Several compounds have been described that block early steps of infection (receptor binding, entry and uncoating of the virus). Other experimental drugs have been described that inhibit

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translation of the picornavirus RNA or genome replication (for a review, see Carrasco, 1994). In contrast, only a very small number of compounds have been described that inhibits the poliovirus assembly or morphogenesis. Among them are 2-amino-4,6-dichloropyrimidine (Py 11) and 2-amino-5-(2-sulfamoylphenyl)-1,3,4-thiadiazole (G 413). Py 11 reversibly prevents the assembly of the P1 proteins (the precursor of the structural proteins) into 14S particles (La Colla et al., 1976, 1977). G 413 probably prevents the assembly by interacting directly with the structural proteins (Bonina et al., 1982).

Recently, it has been shown that 5-(3,4-dichlorophenyl) methylhydantoin (referred to here as hydantoin) possess anti-picornavirus activity, specifically against the three serotypes of poliovirus, several coxsackieviruses and a less pronounced activity against human rhinoviruses (Gerzon et al., 1974; Vance et al., 1997). The mode of action of hydantoin against poliovirus (the best-studied model virus of the picornaviruses) has been studied in detail. It was shown, that hydantoin is an inhibitor of the morphogenesis of the virus (Vance et al., 1997).

Poliovirus morphogenesis can be described as a stepwise assembly process (Putnak and Phillips, 1981; Hellen and Wimmer, 1992). In this cascade of events, hydantoin affects at least one of these assembly steps. In hydantoin-treated cells a new putative assembly intermediate (110S) could be detected. As this intermediate has properties consistent with that of a packaging intermediate, it has been suggested that hydantoin inhibits the encapsidation of viral RNA (Vance et al., 1997).

Molla et al. (1991, 1993) has developed a cell-free system for de novo synthesis of infectious poliovirus. This cell-free system is an ideal tool to study the assembly of poliovirus. The cell-free system consists of an extract of uninfected HeLa cells and is programmed with viral RNA. Consequently, there is direct access to the replication machinery, unhampered by intact cellular membrane(s). As no extraction procedure is required for the detection of the assembly intermediates, the isolation and characterisation of such particles is relatively easy. Moreover, assembly intermediates can be studied without membrane passage. Making use of these properties, we have studied the mechanism of action of hydantoin in this cell-free system.

2. Materials and methods

2.1. Cells and virus

HeLa S-3 cells in suspension were grown in SMEM medium, supplemented with 0.03% glutamine and 5% calf serum.

Purified type 1 (Mahoney) poliovirus was used throughout the experiments. All virus purification procedures were as previously described (Everaert et al., 1989).

2.2. Drug solutions

5-(3,4-dichlorophenyl) methylhydantoin was kindly obtained from Lilly Research Laboratories. Stock solutions (10 mg/ml) of the product were made in dimethyl sulfoxide.

2.3. Preparation of vRNA

The vRNA was extracted from purified poliovirus (Mahoney strain) using an RNA isolation kit (PUREscript, BIOzym). In a sterile tube, 1250 μl cell lysis solution (PUREscript, BIOzym) was added to 500 μl purified virus (100 μg/100 μl, see Section 2) and mixed by vortexing for 5 min. Afterwards, 500 μl protein-DNA precipitation solution (PUREscript, BIOzym) was added and mixed by gently inverting the tube 10 times. The mixture was cooled on ice for 5 min and centrifuged for 15 min at 13 000 $\times g_{max}$ and 4°C in a centrikon A8.24 rotor. The RNA containing supernatant was collected and centrifuged for 15 min at 13 000 $\times g_{max}$ and 4°C in a centrikon A8.24 rotor. To the supernatant, 1 μl of a 20 mg/ml glycogen solution and 1500 μl of isopropanol were added. The tube was gently inverted 50 times and centrifuged for 15 min at 13 000 $\times g_{max}$ and 4°C in a centrikon A8.24 rotor. The supernatant was poured off and the vRNA pellet was washed with 1500 μl 70% ethanol. Finally, the vRNA was collected by centrifuga-

tion; air dried and resolved in 50 μl diethyl pyrocarbonate treated water (RNase free water). The concentration and the A_{260}/A_{280} ratio of the vRNA preparations were spectrophotometrically measured and the ratio was found to be ~ 1.9, as expected for pure vRNA. The vRNA was stored at -80°C. Before use, the integrity of the vRNA was determined by agarose gel electrophoresis (0.8%).

2.4. Preparation of cytoplasmic HeLa extract

Cytoplasmic HeLa extract (HeLa S-10) was prepared as previously described (Molla et al., 1991, 1993; Cuconati et al., 1998). Briefly, HeLa cells were harvested en washed with HBSS buffer. Afterwards, the cells were resuspended in a hypotonic buffer (10 mM K-HEPES, pH 7.4, 10 mM potassium acetate, 1.5 mM magnesium acetate and 2.5 mM dithiothreitol), cooled on ice for 10 min and lysed with a Dounce Homogenizer. The degree of lysis was determined visually by phase-contrast microscopy. Cell debris and nuclei were removed by centrifugation and the supernatant was dialysed against 2 l of dialysis buffer (10 mM K-HEPES, pH 7.4, 90 mM potassium acetate, 1.5 mM magnesium acetate and 2.5 mM DTT). The dialysed lysate was centrifuged and 50% glycerol was added to a final concentration of 10%. After treatment with a micrococcal nuclease to remove the endogenous mRNA's, the cytoplasmic HeLa lysate was aliquoted and stored by -80°C.

2.5. Cell-free synthesis of poliovirus

The preparations of the cell-free systems were carried out as described previously (Molla et al., 1991, 1993; Cuconati et al., 1998) with slight modifications.

Rabbit Reticulocyte Lysate (10%) (Promega) and pirodavir (10 μg/ml), a stabiliser of the viral capsid synthesised by the Janssen Research Foundation (Rombaut and Boeyé, 1991; Rombaut et al., 1994), was added to each cell-free system (Verlinden et al., in preparation). These additives enhanced the efficiency of the cell-free system. The final volume of each cell-free system was 25 μl, including 17.5 μl mastermix (containing 55% cytoplasmic HeLa lysate, 1.5 mM ATP, 296.5 μM GTP, 284 μM CTP and UTP, 14.8 mM creatine phosphate, 37 μg/ml creatine phophokinase, 28 mM K-HEPES pH 7.4, 37 μg/ml calf liver tRNA, 1.85% amino acid mix (minus methionine), 370 μM spermidine, 545 μM magnesium acetate, 1.31 mM magnesium chloride, 159 mM potassium acetate, 88 μCi tran^{35}S-label™), 1 μl 250 μg/ml pirodavir solution, 2.5 μl Rabbit Reticulocyte lysate and 4 μl DEPC treated water, vRNA and other reagents. Incubation at 34°C for 15 h was followed by an RNase treatment.

2.6. Analysis of labelled proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE was essentially as described by Laemmli (1970) with the following modifications. After denaturation, the proteins were analysed by SDS polyacrylamide gelelectrophoresis in 12.5% slabgels (0.1% SDS). Gels were fixed in an acetic acid–methanol–water (10/45/45) solution, fluorographed with Amplify (Amersham), dried and exposed to BioMax MR-1 film (Kodak) at -80°C.

2.7. Analysis of assembly intermediates by sucrose gradient ultracentrifugation

In each sample, free ^{35}S-methionine was removed by dialysis. For the analysis of the procapsids (74S) and the virus (160S), the dialysed samples were layered onto a 15–30% sucrose gradient in PBS buffer (137 mM NaCl; 2.7 mM KCl; 8.1 mM $Na_2HPO_4 \cdot 12H_2O$; 1.4 mM $NaH_2PO_4 \cdot H_2O$ pH 7.4) and centrifuged for 2 h 15 min at $180\,000 \times g_{av}$ and 4°C in a centrikon TST 41.14 rotor. For the analysis of the 5S and 14S particles, dialysed samples were layered onto a 5–20% sucrose gradient in PBS buffer and centrifuged for 17 h at $55\,000 \times g_{av}$ and 4°C in a MSE SW 30.3 rotor. After centrifugation, 400 μl fractions were collected for analysis of the radioactivity.

2.8. Plaque assay

Human Embryonal Kidney (HEK) cells in monolayer were grown in 55 mm diameter petri dishes. Serial dilutions of each sample were prepared in SMEM medium. From each dilution, 250 μl was added to a petri dish. After 30 min at room temperature, 5 ml of a first overlay (MEM medium, supplemented with 1.1% agar and 10% calfserum) was added. After the petri dishes were incubated for 2 days at 37°C, the HEK cells were fixed with 5 ml of 10% formaldehyde for 2 h at room temperature. The first overlay was removed and the cells were stained with a crystal violet solution (0.2% crystal violet, 2% ethanol, and 10% formaldehyde in water) for 1 h. The cells were washed with water and the plaques were counted.

3. Results

3.1. Influence of hydantoin on poliovirus synthesis in the cell-free system

To have a better judgement of the antiviral activity of hydantoin, it was decided to assay the influence of this compound on de novo synthesis of infectious poliovirus in a cell-free system. As explained in detail in Section 2, the cell-free system consists of an extract of uninfected cells which is programmed with poliovirus RNA. As there is direct access to the replication machinery, such a cell-free system is very useful to study the influence of antiviral compounds on viral RNA replication, protein synthesis and the post-synthetic cleavages and, last but not least, the assembly of the virus. Moreover, the particular cell-free system described here is very useful for studying antiviral compounds acting on the very last steps of the poliovirus replication cycle. The reason is that in the infected cell, there is a nearly perfect synchronisation between RNA synthesis, protein synthesis and assembly of the virus (Koch and Koch, 1985, and references cited therein) (our observations). In the cell-free system, in contrast, there is a dissynchronisation of the different replication steps of the virus. Consequently, there exist time-windows in which the influence of an antiviral compound on only one of the replication steps of the virus can be studied.

Specifically, (Verlinden et al., submitted for publication):

1. upon programmation of the cell-free system with vRNA, viral protein synthesis is reaching extremely fast a maximum at 2 h post-programmation (p.p.). Post-synthetic cleavage of the precursor proteins is also very fast. Consequently, the bulk of capsid proteins is already synthesised 2–3 h p.p. Therefore, during this time-window, the influence of an antiviral compound on the protein synthesis and the post-synthetic cleavages can be studied;
2. the infectivity (and assembly) of the virus, starts only 6–7 h p.p. and reaches a maximum 9 h p.p., due to the later start of the RNA synthesis. During this time-window, the influence of an antiviral compound on viral RNA synthesis and the assembly can be studied. If an antiviral compound has no inhibitory activity on RNA synthesis, which is the case with hydantoin (Vance et al., 1997; and our observations), the activity on assembly can be studied exclusively.

The influence of hydantoin on the replication of poliovirus in the cell-free system was tested as follows. Hydantoin was added in several concentrations (5, 20 and 50 μg/ml, respectively) either at the moment of programmation (before protein synthesis) or at 3 h p.p., before the assembly starts. This concentration range of hydantoin was chosen, because Vance et al. (1997) showed that the antiviral effect of hydantoin in the cell was significant only at 50 μg/ml. Two lower concentrations were included, to assess whether the easy access of the drug (no membrane passage in the cell-free system) may lower the maximal inhibitory concentration of the drug. ^{35}S Radiolabel was added at the moment of programmation. At 15 h p.p., total protein synthesis (total incorporation of radiolabel) was determined and found to be identical in all samples, regardless of the presence or absence of the drug (results not shown). Portions were analysed by SDS-polyacrylamide gels (12.5%). In the absence of hydantoin all viral proteins were visible (Fig. 1, lane 3), with the exception of capsid proteins VP2 and VP4, al-

though upon longer exposure a band was visible on the VP2 position. When hydantoin was added immediately after programmation, only high molecular proteins were visible (Fig. 1, lanes 4–6). In addition to capsid precursor P1, it appeared as if the precursors of the non structural proteins of the P3 region were present in excess. Moreover, at the top of the gel a very large protein was present, which we speculate is the uncleaved polyprotein P1P2P3. The capsid proteins VP0, VP1, VP3 and all other non capsidial proteins are formed in a low amount in the presence of hydantoin, compared to precursors of these proteins. In other words, the post-synthetic cleavage of the precursor proteins is strongly inhibited due to the addition of the different concentrations of the hydantoin. In contrast, when hydantoin is added at 3 h p.p., a normal cleavage pattern is observed (Fig. 1, lanes 7–9).

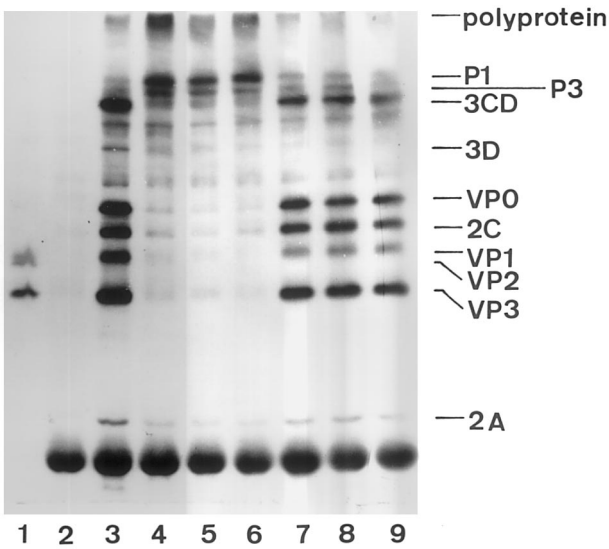

Fig. 1. Influence of hydantoin on poliovirus protein synthesis and post-synthetic cleavages in a cell-free system. Cell-free systems (25 μl) were prepared as described in Section 2. Programmation was with 600 ng of poliovirus RNA (except for one mock programmation, see lane 2), and trans^{35}S-label™ was added. To each cell-free system, hydantoin was added either at the moment of programmation (lanes 4–6) or 3 h post-programmation (lanes 7–9). Three different concentrations of hydantoin were used: 5 μg/ml (lanes 4 and 7), 20 μg/ml (lanes 5 and 8) and 50 μg/ml (lanes 6 and 9). One cell-free system received no hydantoin (lane 3). Incubation for each cell-free system was for 15 h at 34°C. After incubation, all samples were RNase-treated and stored at −80°C until use. Portions of each sample were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, as described in Section 2. Lane 1: Poliovirus capsid proteins.

Those samples in which a normal cleavage pattern was observed in spite of the drug (see Fig. 1, lanes 7–9), as well a control sample (no addition of hydantoin) were submitted to sucrose gradient ultracentrifugation (Fig. 2). In all samples viral intermediate assembly particles were found. However, compared to the control sample (closed circles; no addition of hydantoin), no 160S virions and few procapsids (74S) were found (see Fig. 2(B)). When hydantoin was added 3 h p.p. (only the results for hydantoin 20 μg/ml are shown, but both other concentrations showed comparable results), the bulk of viral proteins remained in the 5S area (see Fig. 2(A); open circles).

The de novo synthesis of virions was also measured in a plaque assay. Whereas in the absence of hydantoin 2.7×10^7 PFUs per millilitre of incubation mixture were found, no infectivity was observed in the presence of hydantoin, irrespectively of the time of addition. Of course, if hydantoin is added immediately after programmation, no capsid proteins are synthesised and particle assembly cannot be expected. However, if hydantoin is added to the extract after the bulk of proteins are synthesised and the post-synthetic cleavages occurred (3 h p.p.), capsid proteins VP0, VP1 and VP3 are formed, but they are deficient in assembly (Table 1). As a consequence, de novo synthesis of infectious virus was not observed in the presence of the three different, tested concentrations of hydantoin.

The observation of this double action of hydantoin on poliovirus replication in the cell-free system, prompted us to investigate whether both phenomena also occurred in the infected cell.

3.2. Activity of hydantoin in the infected cell

Cells were infected with 10 PFU per cell of Mahoney virus, and hydantoin was added 1 h p.i. at different concentrations (20, 50 and 100 μg/ml, respectively). These concentrations fall between the MIC_{50}, which is ~20 μg/ml for poliovirus type 1 proliferation and the TD_{50} on HeLa S-3

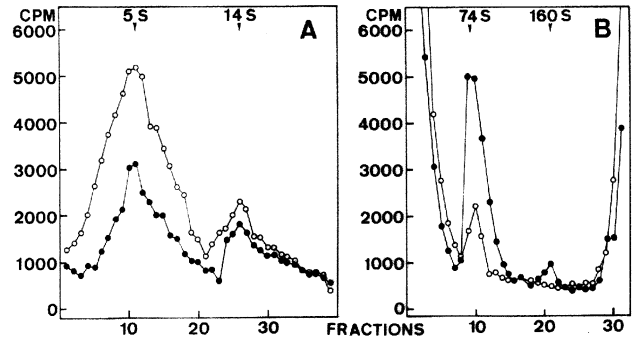

Fig. 2. Sedimentation analysis of poliovirus related particles synthesised in a cell-free system, influence of hydantoin. Cell-free systems (25 μl) were prepared as described in Section 2. Programmation was with 600 ng of poliovirus RNA and tran^{35}S-label™ was added. One cell-free system received 20 μg/ml of hydantoin at 3 h post programmation (○), whereas in the other cell-free system there was no addition of hydantoin (●). After incubation for 15 h at 34°C, both samples were RNase-treated and dialysed to remove free radiolabel. Equal portions of each sample were either layered onto a 5–20% sucrose gradient for separation of 5S from 14S particles (A) or onto a 15–30% sucrose gradient for separation of 74S from 160S particles (B). Gradients were centrifuged, fractionated and counted as described in Section 2.

cells, which is 200 μg/ml (results not shown). Radiolabel was added to the culture at 3 h p.i. when the bulk of viral protein and RNA synthesis occurs (there is a nearly perfect synchronisation of protein synthesis and viral replication in the cell). Cells were harvested at 6 h p.i. (at the end of viral replication), lysed and nuclei and celdebris were removed. Total protein synthesis was measured and found not to be influenced by the addition of hydantoin, except for the highest concentration of hydantoin, where a twofold decrease was determined (results not shown). Portions of each cell extract were analysed by SDS-polyacrylamide gelelectrophoresis. As shown in Fig. 3, hydantoin also affects the post-synthetic cleavages in the infected cell. In the absence of hydantoin a typical profile of virus-specific polypeptides was found (see Fig. 3, lane 2). In contrast, in the presence of hydantoin, some major changes (compared to the protein profile in absence of hydantoin) occurred: (1) no VP2 was found for hydantoin concentrations higher than 20 μg/ml (see Fig. 3, lanes 4–5); (2) the amount of the other capsid proteins VP1 and VP3 is decreased; (3) large molecular weight precursors and possibly the polyprotein P1P2P3 occur at the top of the gel. We conclude that the postsynthetic cleavages of the viral proteins in infected cells are also affected upon addition of hydantoin, although the effect may not be as drastic as observed in the cell-free system.

In order to study the activity of hydantoin on the poliovirus assembly, portions of cell extracts were submitted to sucrose gradient ultra centrifugation analysis. Results are summarised in Table 2. In the control experiment (no addition of hydantoin), radioactivity was evenly distributed over virions (160S), procapsids (74S), 14S subunits and 5S particles. At a concentration of 20 μg/ml hydantoin only a small (and maybe not significant) decrease in 160S virions was found. At 50 μg/ml hydantoin, no visible 160S virions were synthesised, this is compensated by an increase of the amount of the radioactivity in the 5S region. At

Table 1
Influence of hydantoin on the different replication steps of poliovirus morphogenesis in the cell-free system[a,b]

Replication step	Time of addition of hydantoin		
	None	0 h p.p.	3 h p.p.
Viral protein synthesis	+	+	+
Post-synthetic cleavages	+	−	+
Assembly	+	−	−
Infectivity	+	−	−

[a] +, no influence.
[b] −, inhibition.

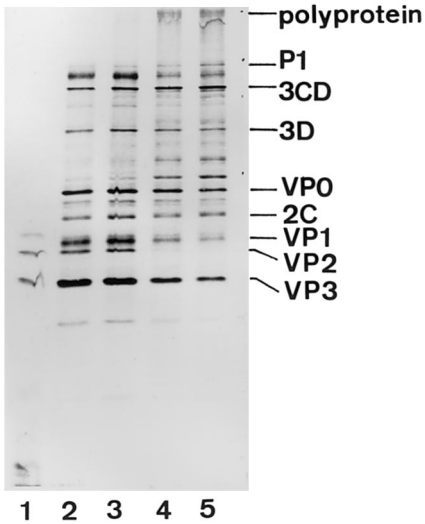

Fig. 3. Influence of hydantoin on poliovirus protein synthesis and post-synthetic cleavages in the infected cell. HeLa suspension cells were infected with type 1 poliovirus (Mahoney strain). At 1 h post infection (p.i.), hydantoin was added in different concentrations: 20 μg/ml (lane 3), 50 μg/ml (lane 4) or 100 μg/ml (lane 5). In one sample there was no addition of hydantoin (lane 2). Radiolabel was added 3 h p.i. and harvesting of the cells was 6 h p.i. Lysates were prepared and analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, as described in Section 2. Lane 1: Poliovirus capsid proteins.

this concentration, where no toxic effect on the cells was observed, the concentration of 74S procapsids and 14S subunits is not decreased. At 100 μg/ml hydantoin, now also the 74S peak decreases, this is further compensated by an increase of the amount of the radioactivity in the 5S region. At this concentration, there is already an effect on the total protein synthesis. This suggests that assembly of the protomer (or P1) and other poliovirus intermediates becomes affected. On the other hand, the formation of 110S particles, as shown by Vance et al. (1997), was not observed, independently of the concentration of hydantoin. We conclude that poliovirus assembly is affected by the addition of hydantoin in the cell-free system as well as in infected cells.

4. Discussion

The replication cycle of poliovirus can be divided into an 'early' phase, comprising attachment, penetration and uncoating of the virus and a 'late' phase involving translation of the RNA into a polyprotein, a cascade of post-synthetic cleavages, the synthesis of progeny RNA, and, in the final stage, the intracellular assembly and release of progeny virions. Whereas the individual steps of the early phase can only be studied by infections of intact cells with virions, the replication steps of the late phase (with the exception of the virus release) and even genetic recombination can be studied in a cell-free system (Barton et al., 1995; Barton and Flanegan, 1997; Duggal et al., 1997; Tang et al., 1997; Cuconati et al., 1998; Duggal and Wimmer, 1999). This cell-free system first developed by Molla et al. (1991, 1993) consists of an extract of uninfected HeLa cells and is programmed with viral RNA. As there is direct access to the replication machinery unhampered by intact cellular membranes, the cell-free system provides an ideal tool to study the behaviour of

Table 2
Influence of hydantoin on in vivo morphogenesis

Hydantoin concentration (μg/ml)	Distribution of radioactivity over polio particles (in percentage)[a]			
	160 S	74 S	14S	5 S
None	28	26	17	29
20	26	29	14	31
50	0.2	28	18	54
100[b]	0.08	14	17	69

[a] Radiolabeling is with trans^{35}S-label™ and separation is by sucrose gradient ultracentrifugation analysis (see Section 2).
[b] Total protein synthesis is decreased with a factor two.

antiviral compounds potentially directed against one of the replication steps of the late phase. Moreover, the cell-free system used here has another particular advantage for the study of potential antiviral compounds. Whereas in the cell infected with poliovirus the steps of viral protein synthesis, viral RNA synthesis and assembly of the virus proceed with nearly perfect synchronisation (Koch and Koch, 1985, references therein and our observations), in the cell-free system there is a dissynchronisation between viral protein synthesis (early in the replication cycle) and the viral RNA synthesis (late in the replication cycle) (Verlinden et al., in preparation). This finding indicates that there are several time-windows (after programmation of the cell-free system with vRNA) in which distinct replication steps and the influence of antiviral compounds on these steps can be studied (Verlinden et al., in preparation).

Hydantoin is one of the first antiviral compounds to be evaluated in the cell-free system. Vance et al. (1997) have previously shown that hydantoin has no inhibitory effect on the early phase of the poliovirus replication cycle, has no influence on viral RNA synthesis, but interfered with the encapsidation of the viral RNA and consequently affects the assembly of poliovirus. Here we provide evidence that the addition of hydantoin to the cell-free system at the moment of programmation resulted in the inhibition of the post-synthetic cleavages (Fig. 1), a phenomenon which can also be seen in the infected cell, albeit it may be less pronounced (Fig. 3). This discrepancy between our data and those of Vance et al. (1997) might be due to an inhibitory effect of cell membrane to hydantoin passage in cell infections or cell compartmentalisation of replication steps in vivo (Pfister et al., 1995).

However, when hydantoin was added to the cell-free system after the viral proteins were formed, but before the assembly starts, a pronounced effect on the assembly process was observed. This observation was also made in infected cells. In general, each assembly step of the morphogenesis is affected. This also confirms the observations of Vance et al. (1997), namely, that hydantoin is an inhibitor of poliovirus assembly. However, neither in cell infections nor in the cell-free system, we observed 110S particles. This new putative assembly intermediate, which has all the properties of a package intermediate (contains vRNA, capsid protein VP0 is partially cleaved in VP2 and VP4), was for the first time observed in hydantoin-treated infected cells (Vance et al., 1997).

In conclusion, the experiments in the cell-free system confirm that hydantoin is an inhibitor of poliovirus assembly. It has been shown that hydantoin has also an inhibitory effect on the post-synthetic cleavages of poliovirus. This effect is more pronounced in the cell-free system than in the infected cell and already at a lower concentration. One hypothesis to explain both effects of hydantoin on poliovirus replication is that hydantoin binds to the uncleaved polyprotein and individual downstream precursor proteins. As a result, the cleavage of these precursors is delayed, if not completely inhibited. If, however, structural proteins have already been formed, the assembly of the virus is affected because the compound remains bound to the capsid protein(s) and causes steric hindrance.

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