Programming of Poliovirus Inhibition of Deoxyribonucleic Acid Synthesis in HeLa Cells

W. W. ACKERMANN AND D. WAHL
Department of Epidemiology and Virus Laboratory, School of Public Health, The University of Michigan, Ann Arbor, Michigan

Received for publication 21 May 1966

ABSTRACT

ACKERMANN, W. W. (The University of Michigan, Ann Arbor), AND D. WAHL. Programming of poliovirus inhibition of deoxyribonucleic acid synthesis in HeLa cells. J. Bacteriol. 92:1051–1054. 1966.—Deletion of arginine from a culture medium reduced the rate of deoxyribonucleic acid (DNA) synthesis in uninfected HeLa cells. The normal rate was promptly restored by addition of arginine. Deletion of arginine also prevented poliovirus from inhibiting DNA synthesis in HeLa cells. However, the inhibitory potential of the infection and the capacity of the host cell for stimulation with regard to DNA synthesis were both retained in arginine-depleted cells which were infected. Upon addition of arginine late in the infection, DNA synthesis was first stimulated and then inhibited.

The level of incorporation of thymidylate into deoxyribonucleic acid (DNA) of HeLa cells in the presence of graded concentrations of arginine in the culture medium was reported previously (1). HeLa cells depleted of arginine, by removal of it from the culture medium for 16 hr, still synthesized DNA at 10 to 20% of the normal rate. The normal rate could be promptly restored by addition of arginine. Deletion of arginine also prevented poliovirus from inhibiting DNA synthesis in HeLa cells. Under these conditions, infection may actually increase the rate of DNA synthesis, depending upon the multiplicity of infection and point of observation in the infectious sequence. The rate of viral replication is not significantly affected by a reduction in arginine. The concentration of amino acid required for viral replication, for virally induced inhibition of synthesis of DNA, and for normal rates of synthesis of host-cell DNA increases in the order listed (1).

The object of the experiments described here was to learn, by the use of shifts in arginine concentrations: (i) whether the capacity of the host cell to be stimulated in regard to DNA synthesis is retained in an infected cell when virally induced inhibition of DNA synthesis is prevented; and (ii) whether the capacity of the infection to inhibit DNA synthesis is retained when this capacity is not allowed expression at the time for which it is usually programmed in the infectious sequence.

MATERIALS AND METHODS

Cells. HeLa cells were grown in monolayers in Roux bottles at 37 C with Eagle's (5) basal medium (twice concentrated) supplemented with 10% calf serum. Cultures were passaged every 7 days. During the experimental period, the medium was replaced with one modified with regard to serum, amino acid, and nucleotide content, as described in Results. Periodically, cells and fluid were inoculated into special media to ensure that they were free from mycoplasma and bacteria (3).

Virus. The Mahoney strain of type 1 poliovirus was passaged routinely in HeLa cells, washed, and concentrated by centrifugation, and used as a suspension in phosphate-buffered saline (PBS, pH 7.0). The virus was assayed, by use of a plaque assay, on monolayers of HeLa cells in 2-oz prescription bottles, and concentration was expressed in plaque-forming units (PFU).

DNA. DNA was extracted from HeLa cells by the method of Colter et al. (4), and isolated from the aqueous phase after precipitation of ribonucleic acid (RNA; with 20% ethyl alcohol, v/v) as described by Martinez-Segovia et al. (7). DNA so isolated was dissolved in saline, precipitated with cold trichloroacetic acid, and dissolved in another portion of the same acid with heating; it was then used to determine the incorporation of H-thymidylate. The DNA was quantitatively determined by the method of Burton (2).

Radioactivity. For measurement of radioactivity, 0.1 to 0.5 ml of sample was added to an appropriate volume of scintillation fluid (80 g of naphthalene, 5 g of 2,5-diphenyloxazole, 50 mg of X-naphthylphenyl-
RESULTS

Effect of addition of arginine upon DNA synthesis in ordinary and infected cells both previously depleted of arginine. The growth medium (60 ml) was removed from Roux bottles containing monolayers of HeLa cells and replaced with Eagle's medium (two times concentrated) from which arginine was deleted; the medium was supplemented with 1% calf serum and 4 \( \mu \)g/ml of each of the deoxyribonucleotides of DNA.

In these and related studies of the mechanism of amino acid control of DNA synthesis, all four deoxyribonucleotides of DNA are added to the culture medium. This provides the most proximal precursors of DNA, and thus ensures the amino acid effect is not merely that of a nucleotide precursor. The nucleotides are used rather than the nucleosides, because, in this system, the latter, even in combination, were found sometimes to depress thymidine incorporation, whereas the former increased the rate of incorporation 20 to 30% over the unsupplemented system. Further, it was desirable that the present condition be identical with previous studies (1), to which these results can be related.

After 16 hr at 37 C, poliovirus was added to some cultures, and others were retained as controls. At appropriate times after addition of virus, arginine (50 \( \mu \)g/ml) was added to some cultures, and \( \mathrm{H}^3 \)-thymidylate (10 \( \mu \)c per culture) was added to all. The experiment was terminated 1 hr after addition of the isotope. DNA was isolated, and the incorporation of \( \mathrm{H}^3 \)-thymidylate was determined. Data from three experiments of similar design are recorded in Fig. 1, wherein the multiplicities of viral exposure, time of additions, intervals of isotope labeling, and rates of isotope incorporation are given.

Addition of arginine to a depleted HeLa cell culture at the 1st through the 5th hr after infection produced a stimulation in the rate of DNA synthesis during the hour interval after the addition. The increase was comparable with that seen in uninfected cells. Thus, during the 4- to 5-hr interval in experiment 1, the DNA synthesis of the infected cell, expressed as counts per minute per microgram of DNA, was stimulated from 20.1 to 27.4 and the uninfected from 16.2 to 27.7. In the same interval during experiment 3, the infected cell showed an increase of 15.9 to 41.3, whereas the uninfected rose from 15.8 to 32.2.

When DNA synthesis was measured during the second 1-hr interval after addition of arginine, the stimulation of the uninfected cell was greater than during the first 1-hr interval after addition. Thus, in experiment 3, the rate of DNA synthesis rose in the 1st hr from 13 to 38.2, and then to 61.4 in the 2nd hr. Further, these uninfected cultures reacted uniformly to addition of arginine at various times throughout the experimental period. Thus, the effect of arginine addition at the 2nd, 3rd, or 4th hr produced (experiment 2) comparable effects upon DNA synthesis measured in the corresponding second 1-hr intervals, i.e., 3 to 4 hr, 4 to 5 hr, 5 to 6 hr, namely, an increase from 11.3 (counts per minute per microgram of DNA) to 60.5, 51.8, and 62.8, respectively.

Such was not the case with the infected cell. Addition of arginine at the 3rd hr (experiment 3) increased the rate in the 4- to 5-hr interval to a lesser degree than the control, 15.9 to 23.8 compared with 15.8 to 61.4. Further, the effect in the second hour intervals after arginine addition was dependent upon the interval of the infectious sequence observed. Addition at 2, 3, or 4 hr (experiment 2) gave values in the 3- to 4-, 4- to 5-, and 5- to 6-hr intervals of 24.4, 9.7, and 4.1, compared with 10.1 for the infected control. Thus, in each second hour interval, inhibition was observed relative to the uninfected control and, in the 5- to 6-hr interval, relative also to the infected control which did not receive added arginine.

Addition of arginine to an infected culture in which virally induced inhibition has been prevented by reduction in the arginine concentration stimulated DNA synthesis during the 1st hr after addition (even in the 5th hour after infection) and inhibited it in the 2nd hr. This was demonstrated clearly in experiment 3, where arginine addition at 3 hr increased the rate in the infected culture from 15.9 to 48.4 in the 3- to 4-hr interval and to 23.8 in the 4- to 5-hr interval, whereas addition at the 4th hr increased the rate to 41 for the latter interval.

DISCUSSION

Poliovirus inhibition of DNA synthesis in HeLa cells occurs only after the 3rd hr postinfection and progresses to near completion by the 5th hr. However, with multiplicities of infection less than 40 PFU per cell, a full complement of amino acids must be maintained at a massive concentration (as in Eagle's medium) in the medium for inhibition. Inhibition of DNA synthesis is prevented by deletion of a single amino acid (e.g., valine, histidine, or arginine) or the presence of canavanine, an analogue inhibitor of arginine. The latter will prevent virally induced inhibition at the 2nd hr but not the 3rd. These
FIG. 1. Effect of the time of addition of arginine on the rate of synthesis of DNA in ordinary and poliovirus-infected HeLa cells which were both depleted of arginine prior to the experimental period. Times of addition of arginine (50 μg/ml) and H³-thymidylate (10 μc per culture) are indicated by the position of A or T, respectively. The intervals of exposure to A and T are indicated by the length and position of the bars. The incorporation of H³-thymidylate into DNA, expressed in counts per minute per microgram of DNA, is recorded above the appropriate bars, indicating the period of isotope labeling. The multiplicities of exposure, expressed as plaque-forming units of poliovirus per cell, are: experiment 1, 14; experiment 2, 52; experiment 3, 24.
previous observations suggested that the inhibition was mediated through synthesis of a protein, which begins between the 2nd and 3rd hr post-infection (1).

The present data show that when inhibition is prevented by deletion of arginine until the 4th to 5th hr, the information in the system necessary for inhibition is not lost and can be expressed later upon addition of arginine, as would have occurred in the ordinary infection (Fig. 1, experiment 2). The data are consistent with the view that the inhibition of DNA synthesis is programmed to begin between the 2nd and 3rd hr and is opened. The program is not affected by whether there is actual expression of inhibition. The potential could correspond to synthesis and accumulation of messenger-RNA, and the actual expression could be related to protein synthesis. It is also clear that full expression of inhibition occurs after arginine addition, only after a lag phase.

DNA synthesis in the uninfected cell is markedly reduced by deletion of arginine, valine, or histidine from the medium, and subsequently stimulated by addition of the amino acid. The stimulation seems also to involve protein synthesis, since concentrations of puromycin which prevent it also inhibit protein synthesis to the same degree (unpublished data). Under the condition of amino acid deletio 1, the limited capacity of the host cell for DNA replication is not inhibited by the viral infection, and, presumably, the cell's ability to synthesize protein is also preserved until late in the infection. This is indicated by the ability of the infected system to be stimulated with regard to DNA synthesis at least until the 5th hr of the infection sequence. This stimulation is apparent late in the infection only during the hour after addition of arginine, because, after a lag of 1 hr, the viral inhibitory mechanism is expressed.

When virally induced inhibition of DNA synthesis was prevented by alteration of the cultural conditions, the inhibitory potential of the infection and the capacity of the host cell for stimulation of DNA synthesis were both retained while [as shown previously (1)] viral replication proceeded at a near normal rate.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-03876-01 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED