Patterns of Cycloheximide and Puromycin Effect on Interferon Production Stimulated by Virus or Polyribonucleotide in Different Tissues[1] (35265)

YANG H. KE AND MONTO HO

Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Cycloheximide and puromycin, two antibiotics which inhibit protein synthesis by different mechanisms (1–3), have been used extensively to test whether or not synthesis of protein is required for interferon production (4). On the basis of such studies, it is commonly assumed that interferon formation stimulated by viruses requires protein synthesis (4), but there is some question about interferon stimulated by a synthetic double-stranded polyriboinosinic polyribocytidylic copolymer [poly I:C (5)]. Some authors suggest that protein synthesis is required (6, 7), but Younger and Hallum (8) showed in mice and Vilcek (9) showed in rabbit cells that interferon production stimulated by poly I:C may not be inhibited by cycloheximide. The late phase of interferon production was even enhanced. Vilcek postulated the possible existence of an "endogenous inhibitor of interferon production" which is inhibited by cycloheximide (9). More recently Ho and Ke (10) showed that in liver slices obtained from rabbits injected with poly I:C, either puromycin or cycloheximide could effectively inhibit interferon production. In this communication, we report that the effect of inhibitors of protein synthesis on interferon production varies from tissue to tissue, and that certain tissues continue to produce interferon in the presence of cycloheximide not only after they are stimulated by poly I:C but also after induction by a virus.

Materials and Methods. Interferon production was measured as follows (10). First, 100 μg of poly I:C (5) or 10^9 PFU of Newcastle disease virus [NDV (11)] were injected intravenously in 500 g rabbits. Five min later, the rabbits were exsanguinated, organs were surgically removed, 100–200 mg slices were placed in a flask containing 4 ml of Krebs–Ringer balanced salt solution saturated with 95% O_2 and 5 % CO_2. Flasks were incubated at 37° in a shaking incubator for 24 hr to obtain total interferon production, or for shorter intervals to obtain mean rates of production. Where indicated, cycloheximide (50 μg/ml) and/or puromycin (100 μg/ml) was added for the duration of the experiment. Samples containing antibiotics were dialyzed before titration for interferon. NDV does not replicate in this slice system. To titrate interferon (10), 2 ml of serial 4-fold dilutions of a sample were incubated overnight in duplicate rabbit kidney cell cultures which were then challenged with about 100 PFU of vesicular stomatitis virus. Titers in units were based on 50% plaque reduction end points and adjusted by the simultaneous titration of a standard rabbit interferon preparation included in each assay (11). The effect of cycloheximide or puromycin on protein synthesis of tissue slices was measured by a 60-min pulse incorporation of 2 μCi of ^{14}C reconstituted protein hydrolysate (222 mCi/mmole) in 2 ml/flask. Incorporation was stopped by immersing slices in 5% trichloroacetic acid (4°). After homogenization, and washing, precipitable material was dissolved in 1.0 *N* NaOH, and counted in a gas-flow counter (Nuclear Chicago).

Results. Table I shows in a representative experiment the total amount of interferon produced in 24 hr by 4 types of tissue slices from animals preinjected with poly I:C or NDV and the effect of cycloheximide and puromycin on this production. To measure protein synthesis of each type of tissue slice, 1 flask acting as the control contained no antimetabolite, 1 contained cycloheximide, and 1 puromycin. These were pulsed and

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TABLE I. Effect of Cycloheximide and Puromycin on Interferon Production in Tissue Slices from Rabbits Stimulated by Poly I:C and NDV.

	Total interferon production						Protein synthesis	
	Control		Cycloheximide treated		Puromycin treated		^{14}C-amino acid incorp. (% control)	
Tissue	Poly I:C	NDV	Poly I:C	NDV	Poly I:C	NDV	Cyclo.	Puro.
Spleen	240,000[a]	220,000[a]	1[b]	9[b]	4[b]	6[b]	0.5	11.3
Lung	2700	3500	56	92	5	11	1.5	11.6
Liver	12,000	8000	6	1	1	1	5.0	21.0
Kidney	290	500	507	22	76	4	1.8	10.8

[a] Interferon (units/100 mg of tissue).

[b] Interferon produced (% of control values in absence of cycloheximide or puromycin).

counted as described under Methods. To measure interferon production of each type of tissue slice, a similar set of 3 flasks was obtained from an animal treated with NDV, and another set of 3 was obtained from an animal treated with poly I:C. Inhibition of ^{14}C amino acid incorporation by cycloheximide, which in most cases was over 95%, was not significantly different in various tissues. It inhibited poly I:C and NDV stimulated interferon in spleen and liver tissue, but not in lung or kidney. Interferon stimulated by poly I:C was markedly accentuated in kidney slices. The effect of puromycin did not vary as markedly, and there was generally more inhibition of interferon than of amino acid incorporation. Puromycin effectively inhibited interferon production in all tissues except poly I:C stimulated interferon in kidney and perhaps NDV stimulated interferon in lung. Qualitatively similar results were obtained in repeat experiments.

To turn now from total interferon to the kinetics of its production: Figure 1 shows rates of interferon production in spleen, lung, liver, and kidney slices from animals injected with poly I:C and the effect of the antibiotics. Cycloheximide inhibited interferon production in liver slices throughout the production cycle. This is consistent with our previous finding that cycloheximide as well as puromycin inhibits interferon production in this tissue (10). In the spleen, interferon was markedly inhibited early, affecting thereby

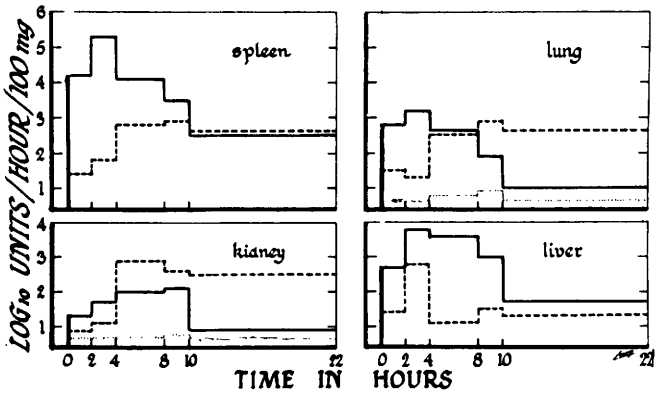

FIG. 1. Effect of cycloheximide on rate of interferon production by different tissue slices from a rabbit injected with 100 μg of poly I:C. Rates of interferon production per hour per 100 mg of tissue are represented on the ordinate on a log scale, to accommodate the great range of rates. (—) interferon production in control flasks; (- - -) effect of cycloheximide (50 μg/ml); (....) flasks containing both cycloheximide and puromycin (100 μg/ml).

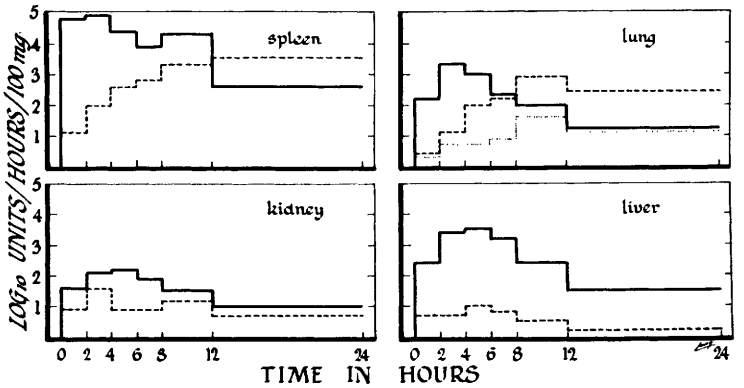

FIG. 2. Effect of cycloheximide on the rate of interferon production by different tissue slices from a rabbit injected with NDV. Rates were obtained as described in the text. (—) interferon production in the absence; (- - -) in the presence of cycloheximide; and (....) effect of both cycloheximide and puromycin.

the total amount produced, but the later phase of production was not inhibited. This pattern was even more apparent in the lung and kidney, such that the total amount of interferon produced under cycloheximide in kidney slices was even greater than in controls (see Table I). However, when lung and kidney tissues were incubated in flasks containing both cycloheximide and puromycin, cycloheximide "resistant" interferon was largely eliminated.

As virus-induced interferon is generally thought to require new protein synthesis, we were especially interested to find that cycloheximide was not very effective in inhibiting NDV-induced interferon from lung and kidney slices (Table I). A study of the rates of production of NDV-induced interferon showed also that the effect of cycloheximide varied with the tissue and the phase of interferon production (Fig. 2). Interferon production was effectively inhibited throughout in liver slices, but in others, there was little or no inhibition of interferon production during the later stages. This was most apparent in the lung. However, here again as in the case of poly I:C-stimulated interferon, the late "escape" was effectively prevented by puromycin.

Discussion. What is the nature of interferon produced in the presence of cycloheximide? Firstly, it may represent direct release of intracellular preformed interferon by a process not requiring protein synthesis. If preformed interferon is released, it should not be affected by any inhibitor of protein synthesis. It has been shown that puromycin inhibited practically all interferon produced by lung or kidney tissue stimulated by either poly I:C or NDV in the presence of cycloheximide. This suggests that the "escaped" interferon production still required protein synthesis. Hence, on the basis of this work and our previous results (10, 12), we find no basis to postulate any interferon production not requiring protein synthesis.

The second possibility is that the "escaped" interferon is synthesized under conditions where a regulatory mechanism has been disturbed by the inhibitor. Recently we reported that actinomycin D, puromycin, and cycloheximide under appropriate conditions could either inhibit or accentuate interferon production in rabbit kidney cell cultures stimulated by poly I:C or by NDV 10- to 100-fold (12). We concluded that all interferon production stimulated by either poly I:C or NDV requires protein synthesis, but that if a control protein normally activated in the later stages of production is inhibited, interferon production may be accentuated. The present data are consistent with this interpretation. The difference in interferon producing patterns in various tissues in the presence and absence of protein inhibitors can be at least partly interpreted in terms of the amount, turnover, or activity of the control protein. The effect of the inhibitors relate

primarily to the later stages of interferon production, which is the period during which the control protein is assumed to be active. It is also apparent that puromycin is less effective than cycloheximide in permitting "escape" of interferon production of slices. Perhaps cycloheximide, which blocks the translocation of peptidyl-tRNA from the aminoacyl site to the peptidyl site on ribosomes may permit more biologically active protein, whether interferon or control molecules, to be formed than puromycin which prematurely terminates the growth of peptide chains (1, 2). Additionally, the control protein may be labile and, in the presence of cycloheximide, it may be unable to reach the concentrations necessary for its action. Hence, under cycloheximide, interferon is made without control and may result in accentuated production. Such a labile negative control molecule has been postulated for tyrosine aminotransferase (13). Finally, while the full explanation of these and other (8, 9) observations is not yet at hand, the different responses of tissues to protein inhibitors suggest that data obtained from animal work (4, 8) may represent the sum of many patterns of responses of various interferon producing tissues, and the absence of a given effect of an inhibitor on interferon production in the animal should no longer be simply interpreted in terms of its primary mode of action.

Summary. Although cycloheximide and puromycin inhibit interferon production by liver and spleen slices from rabbits preinjected with a polyribonucleotide or Newcastle disease virus, production in kidney and lung slices is less affected and may be enhanced. The "escape" of interferon, which we postulate is due to preferential inhibition of a regulatory protein, was primarily in the later phases of production, and could be prevented by a combination of cycloheximide and puromycin.

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