CHANGES IN MECHANISM OF EFFECT OF STROPHANTHIN K ON INTERFERON PRODUCTION IN CELL CULTURES

N. A. Zeitlenok, F. N. Reizin, and E. A. Gorbachkova

UDC 576.858.095.383.095.18:615.22:547.918

The cardiac glycoside strophanthin K, when added to a culture of chick embryonic fibroblasts, inhibits synthesis of cell protein. The drug prolongs the lag-period of interferon synthesis and also the actinomycin-sensitive phase of interferon production induced by influenza B virus, evidently because of inhibition of the beginning of synthesis of messenger RNA for interferon.

The cardiac glycoside strophanthin K, when added (25 μg/ml) to a cell culture not later than 80-90 min after influenza B virus, reversibly depresses the formation of interferon induced by the virus in chick embryonic fibroblasts but has no effect on adsorption of virus by the cells [1].

The object of this investigation was to study the mechanism of the inhibitory action of strophanthin K on interferon production.

EXPERIMENTAL METHOD

Methods of use of cultures of chick embryonic fibroblasts, and methods of obtaining and titrating interferon have been described previously [1]. Synthesis of cell protein was determined quantitatively from the level of incorporation of valine-C^{14} into cell protein. The protein content was determined by Lowry's method [3]. Crystalline strophanthin K (L'vov Pharmaceutical Chemical Factory), actinomycin D (Merck, USA), and 6-azauridine (Spofa, Czechoslovakia) were used in the investigation. The concentration and method of use of the substances are described below.

TABLE 1. Combined Action of Strophanthin K and Actinomycin D on Interferon Production in Culture of Chick Embryonic Fibroblasts

Strophanthin K concentration, μg/ml	Titer of interferon (in PDD_{50}/0.5 ml) in presence of various concentrations of actinomycin D		
	0 μg/ml	0.01 μg/ml	0.1 μg/ml
0	182	140	16
2.5	211	131	0
6.0	16	22	0

Institute of Poliomeylitis and Virus Encephalitis, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. P. Chumakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 10, pp. 66-69, October, 1970. Original article submitted May 11, 1970.

TABLE 2. Effect of 6-azauridine on Action of Strophanthin K in Inhibiting Interferon Production in Culture of Chick Embryonic Fibroblasts

Agents for treating cells infected with interferonogen (20°, 3 h)	Washing cells to remove virus and reagent	Strophanthin K (in μg/ml)	Titer of interferon (PDD_{50}/0.5 ml)
Earle's solution	+	0	1 148
The same	+	25	251
6-azauridine	−	0	380
The same	+	0	1 150
The same	+	25	≤ 12

Legend: −cells not washed; + cells washed before addition of strophanthin K.

TABLE 3. Effect of Strophanthin K on Synthesis of Cell Protein, Weight for Weight, in Culture of Chick Embryonic Fibroblasts

Agent for treating cells (20°, 3 h)	Cells washed before addition of valine-C^{14}	Synthesis of cell protein, weight for weight (in % of control)
Earle's solution	−	100
Control	+	124
Influenza B virus (strain Lee)	−	71
The same	+	124
Strophanthin K	−	55
Strophanthin K+influenza B virus (strain Lee)	−	51
The same	+	123

Legend: − cells not washed; + cells washed before addition of valine-C^{14}.

RESULTS

To discover which stages of interferon formation are sensitive to the glycoside, its effect on the dynamics of interferon production was studied. Contact between the cells and strophanthin at 37° for 2 h, followed by washing and changing the medium, delayed the appearance of interferon in the medium, although its final yield was not reduced (Fig. 1). When the cells were treated at 20°, the times of appearance of interferon in the experimental and control series were identical. These results show that the drug acts on the early stages of interferon production and suggests that strophanthin inhibits the onset of synthesis of messenger RNA (mRNA) for interferon.

To test this hypothesis, the effect of the glycoside on the actinomycin-sensitive phase of interferon production was investigated. Cells were treated with strophanthin at 37 or 20° for 2 h, then washed with Earle's solution and incubated at 37°. After various time intervals, actinomycin D was added to the maintenance medium up to a final concentration of 1 μg/ml, and the cultures were kept at 37° until 17 h after addition of the virus, after which the final titer of interferon was determined. The action of strophanthin on the cells during the first 2 h after addition of virus lengthened the actinomycin-sensitive phase of interferon production (Fig. 2), i.e., shifted the time when synthesis of mRNA for interferon is completed by about 2 h relative to the control. The results of these experiments are in good agreement with data described above for the effect of strophanthin on the dynamics of interferon synthesis, and with the view that strophanthin delays the onset of mRNA synthesis.

Under the experimental conditions used, no synergism could be detected between the action of strophanthin K and of actinomycin D in concentrations of 2.5 and 0.01 μg/ml, respectively, on interferon production when added simultaneously, whereas in doses of 6 and 0.1 μg/ml, each of the substances almost completely suppressed interferon production irrespective of whether the other was added or not (Table 1).

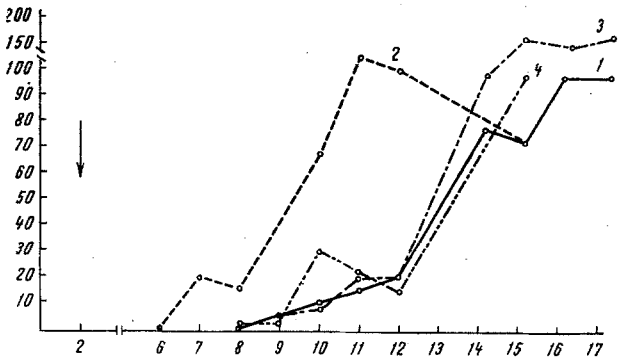

Fig. 1. Effect of strophanthin K on duration of lag-period of interferon synthesis in culture of chick fibroblasts: 1) incubation of cells with Earle's solution for 2 h at 20°; 2) same at 37°; 3) incubation of cells with strophanthin K for 2 h at 20°; 4) same at 37°. Arrow indicates time when cells washed to remove virus and drug. Abscissa, time after infection (in h); ordinate, titer of interferon (in % of control).

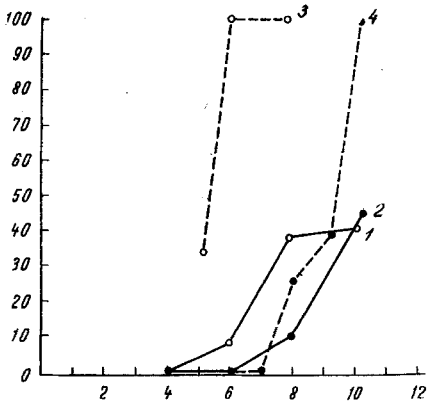

Fig. 2. Effect of strophanthin K on actinomycin-sensitive phase of interferon production in culture of chick fibroblasts. 2 and 4) Cells treated with strophanthin K at room temperature or 37° respectively, for 2 h from beginning of infection, and then washed; 1 and 3) control to corresponding experiments (cells not treated with strophanthin). Abscissa, times of addition of actinomycin D after infection (in h); ordinate, titer of interferon (in % of control).

Detection of delay in the initial stage of RNA synthesis necessary for interferon formation, produced by strophanthin K, suggested that the effect of 6-azauridine on the process be studied. This substance reversibly inhibits RNA synthesis by blocking incorporation of nucleotides [4]. For this purpose, the cell culture was infected with virus and treated with 6-azauridine at 20° for 3 h, then washed, strophanthin was added (25 μg/ml), and the interferon titer was determined 17 h later. In this experiment interferon synthesis was almost completely suppressed, whereas in the control (without strophanthin) interferon titers were reasonable high (Table 2). These facts are further support for the hypothesis that strophanthin inhibits the onset of synthesis of mRNA for interferon, and this, together with the reversibility of its action, readily explains the need for adding the drug to the culture before or after the virus in the early stages and for its presence in the cell medium until and end of the experiment, to allow its activity to be exhibited.

It may be asked how strophanthin prevents the onset of synthesis of mRNA for interferon. Synthesis of DNA-dependent RNA, suppressed by actinomycin, continued under the experimental conditions used for 5-6 h, whereas the glycoside acts only if added in the early periods after infection. Consequently, it has no effect on synthesis of mRNA when this has already begun. It remains to be explained whether strophanthin has any effect on protein synthesis. To solve this problem, experiments were carried out using an isotope label. Treatment of the cells with influenza B virus, strain Lee, in a dose of 3 ID_{50} per cell for 3 h at 20° lowered the synthesis of cell protein, weight for weight, by about 30%. Under the same conditions strophanthin K (25 μg/ml) lowered the protein synthesis by about 50%. When the virus and glycoside acted simultaneously, the level of inhibition remained the same. Suppression of protein synthesis by virus and strophanthin was reversible if the cells were washed before addition of valine-C^{14}.

These experiments showed that strophanthin K can inhibit protein synthesis, but synthesis of the polypeptide chain of interferon is not sensitive to the action of strophanthin, for it begins, under the experimental conditions used, 7 h after infection, and at these times the introduction of strophanthin is ineffective.

It can be assumed that strophanthin acts on synthesis of a protein necessary for interferon production, which takes place during the first 3 h after infection, but the inhibition of this synthesis by p-fluorophenylalanine does not prolong the lag-phase of this process [2], as occurred in experiments with strophanthin. Mechanism of trophanthin inhibition of the onset of synthesis of the mRNA for interferon will be subject of future research.

LITERATURE CITED

1. F. N. Reizin and N. A. Zeitlenok, Byull. Éksperim. Biol. i Med., No. 12, 65 (1969).
2. A. Buchan and D. C. Burke, Biochem. J., 94, 9P (1965).
3. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
4. D. Wilson and P. Logerfo, J. Bact., 88, 1550 (1964).