EFFECT OF STROPHANTHIN K ON INTERFERON PRODUCTION IN A CULTURE OF CHICK EMBRYONIC FIBROBLASTS

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Strophanthin K inhibits interferon formation in a culture of chick embryonic fibroblasts induced by influenza virus B if the cells are treated with it before induction of interferon formation, simultaneously with its induction, and after induction but not more than 80-90 min after the beginning of infection. Interferon formation is restored if the cells are washed to remove the strophanthin.

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There is much information in the literature on the effect of cardiac glycosides on cell phosphorylation [3, 5, 7, 8]. This process is of considerable importance as a source of energy for protein synthesis in general and interferon synthesis in particular.

The object of this investigation was to study the effect of the cardiac glycoside strophanthin K on interferon synthesis in vitro in chick embryonic fibroblasts (CEF).

EXPERIMENTAL METHOD

Influenza virus B (strain Lee) was used to induce interferon synthesis in a dose of 3 ID_{50}/cell in a 6-day CEF culture. After investigation of the culture fluid for interferon activity, the virus contained in it was inactivated at pH 2.0 for 24 h at 4°, after which the liquid was neutralized.

Interferon was titrated in a similar cultured aged 3 days by the method of inhibition of plaque formation by Chikunguniya virus (50-100 PFU/flask). The interferon titer was expressed in PFU_{50} units/0.5 ml [1].

Strophanthin K obtained from the Experimental Factory of the All-Union Pharmaceutical Chemical Research Institute was used in the investigation; 0.4 ml of its solution in a concentration of 25 μg/ml was applied to the CEF monolayer after removal of the culture medium, and kept for 60 min at a temperature of 20°; 8 ml of Eagle's medium in Earle's solution, pH 7.2, was then added and the specimen was incubated at 37° for 24 h. In the control, an equal volume of Earle's solution was added instead of strophanthin.

EXPERIMENTAL RESULTS

To determine the effect of strophanthin K on interferon synthesis, the CEF monolayer was treated with strophanthin at 20° before, at the same time as, or various times after the addition of the interferon inducer. Flasks with CEF, treated with strophanthin at the various times, were placed at the same time in an incubator at 37°. For comparison, cells in a parallel series of cultures were treated with a solution of actinomycin D (2.5 μg/ml) by the same method as with strophanthin.

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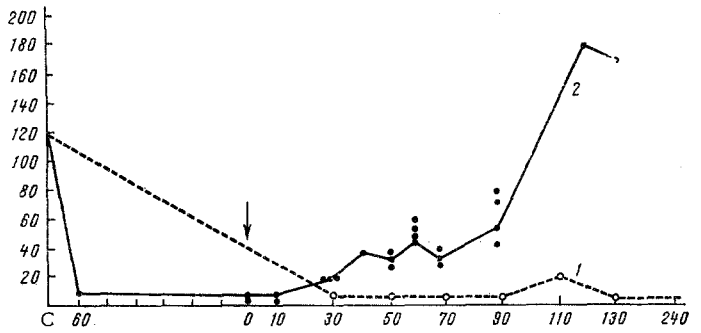

Fig. 1. Action of strophanthin K (25 μg/ml) and actinomycin D (2.5 μg/ml) on interferon synthesis in CEF depending on time of addition of preparation. Abscissa, time (in min) of addition of reagent relative to time of addition of virus (marked by arrow); ordinate, titer of interferon (in PFU_{50}/0.5 ml); 1) actinomycin D; 2) strophanthin K.

TABLE 1. Reversibility of Action of Strophanthin K on Interferon Synthesis in CEF Culture

Time (in min) relative to time of addition of virus (0)		Interferon titer (in PFU_{50}/0.5 ml)
addition of strophanthin K	washing cells	
Not added	Not washed	104
—60	Not washed	18
—60	0	186
0	Not washed	0
0	+60	192
+15	Not washed	0
+15	+75	120

Strophanthin K completely inhibited interferon formation if it was added to the cell culture not later than 80-90 min after the interferon inducer. No correlation was found between the actions of strophanthin K and actinomycin D on interferon synthesis: The latter inhibited interferon formation even when added to the cell culture 4 h after the beginning of infection (Fig. 1).

Strophanthin K, in the same concentration, did not affect adsorption of influenza B virus on the cell culture. From an initial infective titer (in log ID_{50}/0.2 ml) of 5.24, the titer of virus fell after absorption on untreated cells to 4.48, and on cells treated with strophanthin K for 60 min at 20° to 4.16. The hemagglutination titer (in hemagglutination units/0.5 ml) was 128, 32, and 2, respectively. No effect on proliferation of influenza B virus in the allantoic cavity of 11-day chick embryos was observed after injection of 0.4 ml of a solution of strophanthin K (200 μg) into it before infection. This preparation likewise had no effect on adsorption of influenza B virus on human group 0 erythrocytes at different temperatures or on the ability of the erythrocytes to agglutinate under the influence of the virus.

The optimum concentration of strophanthin K to inhibit interferon synthesis was 25 μg/ml, and raising the incubation temperature of the preparation with the cells to 37° did not increase the sensitivity of interferon synthesis to strophanthin.

The action of strophanthin K in inhibiting interferon synthesis was found to be reversible provided that the cells were washed to remove the drug (Table 1). This observation now described does not conflict with published data [6, 8] indicating that cardiac glycosides do not penetrate into the cell, and that their action is limited to the region of the cell membrane. Strophanthin is known to inhibit the activity of membrane transport $Mg^{++}Na^{+}K^{+}$-dependent ATPase with inhibition of active transport of cations and certain amino acids into the cell [3, 5, 7, 8]. The present writers [2] found that active interferon synthesis takes place readily in the absence of exogenous amino acids. It can therefore be postulated that disturbance of active membrane transport of cations into the cell is an important factor in the mechanism of action of strophanthin K on interferon formation. If, however, it is assumed that inhibition of interferon synthesis by strophanthin K is due to the effect of this drug on activity of $Mg^{++}Na^{+}K^{+}$-ATPase, and it is remembered that the action of glycosides is manifested to an adequate degree after incubation for about 40 min with the cells [4], it can be concluded that the phase of interferon synthesis which is sensitive to strophanthin starts about 70 min after the beginning of infection, and continues until about the 110th-120th minute. The action of strophanthin under these circumstances was much shorter in duration than that of actinomycin D.

These results confirm the conclusion of Link and co-workers [6] that interferon production is inhibited by ouabain (strophanthin D), but in contrast to the experiments of these workers, in the present experiments strophanthin K did not disturb absorption of the virus on the cells.

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