ACTION OF DL-19-NOR-D-HOMOTESTOSTERONE ON THE INTERFERON SYSTEM AND ON SYNTHESIS OF TOTAL CELL PROTEIN IN TISSUE CULTURE

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DL-19-nor-D-homotestosterone in a culture of chick embryonic fibroblasts inhibits total protein synthesis. Interferon synthesis induced by influenza virus is inhibited to a greater degree than total protein synthesis. The compound has no effect on activity of exogenous interferon, i.e., on the synthesis of antiviral protein.

Much attention has recently been paid to the study of the antiviral protein interferon, produced by cells in vivo and in vitro in response to the introduction of viruses and certain other agents. A promising method of studying the mechanism of formation and antiviral action of interferon is by the use of substances inhibiting or stimulating these processes. Of special interest from this point of view is the use of biologically active substances such as hormones, although their influence on the synthesis and activity of interferon is the subject of only a few communications [10,13,16].

In the present investigation the substance chosen for study of its effect on interferon production and activity and synthesis of total cell proteins was DL-19-nor-D-homotestosterone* (NDT) [2], which differs from the natural hormone in its greater ability to stimulate protein metabolism in the animal organism [1].

EXPERIMENTAL METHOD

A tissue culture of chick embryonic fibroblasts (CF) was obtained by the usual method. The cells were grown in Eagle's medium. For obtaining interferon, 5-7-day CF cultures were used and infected with active influenza B virus, strain Lee, in a dose of 3 ID_{50} per cell. Interferon activity was determined in the

TABLE 1. Inhibition of Interferon Synthesis by DL-19-nor-D-homotestosterone in a Culture of Chick Fibroblasts

Dose of compound (in μg/ml)	Experiment No.					M	P
	1	2	3	4	5		
0	200	128	64	78	62	106	
50	0	20	18	0	0	8	0,01

Note. Interferon titers are given as PID_{50}/ml (the amount of interferon inhibiting plaque formation of Chikungunya virus by 50%).

TABLE 2. Action of DL-19-nor-D-homotestosterone on Interferon Activity

Dose of compound (in μg/ml)	Dilution of exogeneous interferon		
	1:16	1:64	—
0	0	88	240
1	0	86	Not determined
50	9	160	270

Note. Numbers denote mean number of plaques of Chikungunya virus per flask.

*DL-19-nor-D-homotestosterone (NDT) and D-19-nortestosterone (NT) were obtained from Professor I. V. Torgov and S. N. Ananchenko at the Institute of Chemistry of Natural Compounds of the AN SSSR.

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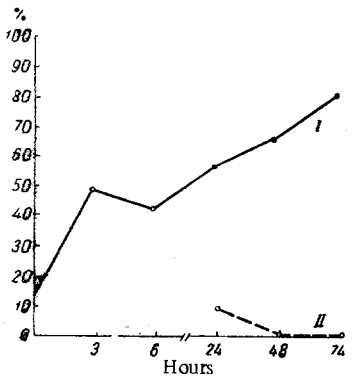

Fig. 1. Effect of duration of incubation with DL-19-nor-D-homotestosterone in concentrations of 50 (I) and 0.1 μg/liter (II) on total protein synthesis in a culture of chick fibroblasts. Abscissa, duration of incubation (in h), ordinate, inhibition of total protein synthesis (in %).

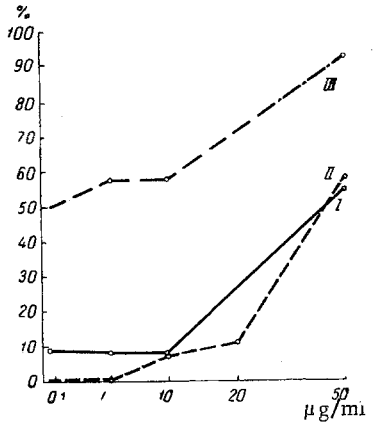

Fig. 2. Effect of concentration of DL- (I) and D-form (II) of 19-nor-D-homotestosterone on total protein synthesis and on interferon synthesis (III) in a culture of chick fibroblasts. Abscissa, hormone concentration (in μg/ml); ordinate, inhibition of total protein synthesis and interferon synthesis (in %).

CF culture by its ability to inhibit plaque formation by Chikungunya virus. Total cell protein synthesis was studied from the level of incorporation of glycine-C^{14} into the cell protein. The protein content was determined by Lowry's method [11].

EXPERIMENTAL RESULTS

The investigations showed that a dose of NDT of 100 μg/ml is the highest dose tolerated by CF cells. The study of the effect of duration of incubation of cells with NDT on total protein synthesis showed (Fig. 1) that addition of NDT in a dose of 50 μg/ml (curve I) simultaneously with the isotope caused some inhibition of protein synthesis, and with an increase in the duration of preincubation this effect increased to reach about 50% by 3 h and 75% by 72 h. Prolonged incubation of cells with small doses of NDT such as 0.1 μg/ml (curve II) did not significantly change the level of cell protein synthesis. An essential condition for exhibition of the inhibitory effect, besides preincubation of the cells with the compound, was its presence in the culture medium during contact of the cells with the isotope. Hence, the anabolic action of the synthetic analog of testosterone not only was not exhibited in tissue culture, but was converted into an opposite action, namely the inhibition of protein synthesis. Similar results have been obtained by the action of other hormones in vitro [5,6,8,15].

To study the effect of NDT on interferon production, the CF cells were incubated with the compound in a concentration of 50 μg/ml for 18-20 h before infection with interferon-inducing virus and for 24 h after infection. Control infected cultures were incubated under the same conditions. As Table 1 shows, in all experiments there was a well-marked statistically significant inhibition of interferon production under the action of NDT. The parallel between the actions of large doses of NDT on total protein synthesis and on interferon synthesis agrees well with published data indicating that interferon synthesis is a process of synthesis of new protein [4,9,19].

The effect of NDT on the antiviral activity of exogenous interferon added to a CF culture preliminarily incubated with the compound was studied under optimal conditions for manifestation of its inhibitory action on total protein synthesis. The results given given in Table 2 show that incubation of the cells with NDT in a concentration of 50 μg/ml reduced the interferon activity only very slightly. In a dose of 1 μg/ml, NDT did not change the interferon activity.

It was also found that NDT, in the highest concentration used, did not possess virucidal properties and did not alter the titer of influenza virus detected in the CF culture 24 h after infection.

The results of the study of the relationship between the inhibitory effect of NDT and NT and their dose are illustrated in Fig. 2. Comparison of the character of curves I and II demonstrates the similarity of the effect produced by the racemate and the D-form of the hormone analog. With an increase in the dose of synthetic hormone analogs, the degree of inhibition of total cell protein synthesis and of interferon synthesis increased. However, small doses of the compounds (0.1-10 μg/ml), not significantly influencing total cell protein synthesis, inhibited interferon synthesis by 50%. The difference between the action of NDT and NT on total cell protein synthesis and on interferon synthesis and activity (interferon-induced synthesis of antiviral protein) thus demonstrated is not peculiar to the action of these compounds alone. The processes we have studied are sensitive to a different extent to

puromycin and to guanidine [3,7,18]; 3-methylcholanthrene [12] and some steroid and growth hormones have different effects on interferon production and on its activity [14,16,17]. Another noteworthy feature is that different preparations do not act in the same direction. With these considerations in mind, qualitative or quantitative differences can be postulated between the protein-synthesizing systems responsible for interferon synthesis, for synthesis of antiviral protein, and for total cell protein synthesis.

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