

EFFECT OF SUBSTANCES MODIFYING THE INTRACELLULAR CYCLIC AMP LEVEL ON INTERFERON FORMATION IN CHICK EMBRYOS OF DIFFERENT AGES

F. N. Reizin, V. M. Roikhel',
and M. P. Chumakov*

UDC 577.19.044: [615.357.
452+615.225.2:547.857.4

Investigation of the effect of adrenalin and theophylline on interferon-synthesis induced in chick embryonic cells by influenza B virus (Lee strain) showed that the action of these substances on interferon production depended on the age of the cell culture. In a one-day culture adrenalin (0.2 $\mu\text{g/ml}$) did not affect interferon formation but theophylline (400 $\mu\text{g/ml}$) stimulated it considerably. In a five-day culture both substances in the same concentrations inhibited interferon synthesis; their action was potentiated if they were given together and it was manifested even if the substances were added 4 h after the beginning of infection of the cells. In the concentration used, the two substances separately or together did not affect proliferation of influenza B virus (Lee strain) in chick embryos, or likewise vesicular stomatitis, Newcastle disease, and Chikungunya viruses in the cells of chick embryos. They had no toxic action on the cells of the tissue culture. The results are discussed from the point of view of the possible effect of the intracellular cyclic AMP level on the synthesis of virus-induced interferon.

KEY WORDS: interferon production; action of adrenalin and theophylline.

There are many data in the literature to show that the mechanism of action of adrenalin and theophylline on cells is connected with their ability to modify the intracellular level of cyclic adenosine-3', 5'-monophosphate (cyclic AMP) [4, 8, 10], a compound of exceptional importance in cell metabolism [2, 3, 11]. Considering the evidence in the literature that exogenous cyclic AMP can modify interferon synthesis [6], it was decided to study the effect of adrenalin and theophylline, which modify the intracellular cyclic AMP level, on interferon synthesis in tissue culture.

EXPERIMENTAL METHOD

The method of preparing the cell culture and of obtaining and titrating the interferon was described previously [1]. Influenza B virus (Lee strain) was used as the interferonogen and vesicular stomatitis virus as the test virus.

EXPERIMENTAL RESULTS

To study the effect of the substances on interferon production a culture of chick embryonic cells (CES) was infected with the interferonogen and the substances for testing were added separately or together to the flask 30 min later. Maintenance medium (Eagle's medium) was then added and the flasks were incubated for 24 h at 37°C.

Adrenalin and theophylline in concentrations of 0.2 and 400 $\mu\text{g/ml}$ respectively inhibited (Fig. 1) interferon formation in the five-day CES; their inhibitory action on interferon production was potentiated when

*Academician of the Academy of Medical Sciences of the USSR.

Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR, Moscow.
Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 79, No. 6, pp. 49-52, June, 1975.
Original article submitted January 28, 1974.

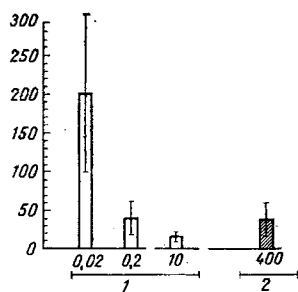


Fig. 1

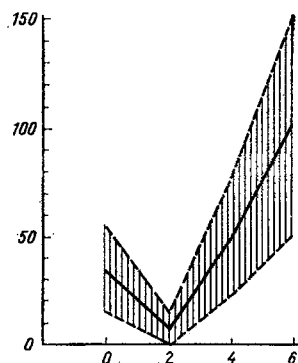


Fig. 2

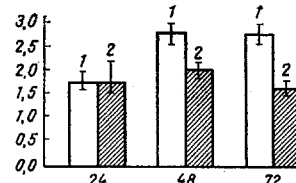


Fig. 3

Fig. 1. Effect of adrenalin (1) and theophylline (2) on formation of virus-induced interferon in chick embryonic cells aged 5 days depending on concentration of substances. Abscissa, concentration of substances (in $\mu\text{g/ml}$); ordinate, interferon titer (in % of control).

Fig. 2. Effect of combined addition of adrenalin (0.2 $\mu\text{g/ml}$) and theophylline (400 $\mu\text{g/ml}$) on interferon formation in chick embryonic cells aged 5 days depending on time of addition of substances. Abscissa, time (in h) of addition of substances after infection; ordinate, interferon titer (in % of control). Shaded zone shows confidence limits.

Fig. 3. Effect of adrenalin on synthesis of virus-induced interferon in chick embryonic cells of different ages. Abscissa, age of cell culture (in h); ordinate, interferon titer (in $\log \text{PID}_{50}/0.5 \text{ ml}$). 1) Control; 2) adrenalin (0.2 $\mu\text{g/ml}$).

TABLE 1. Effect of Adrenalin (0.2 $\mu\text{g/ml}$) and Theophylline (400 $\mu\text{g/ml}$) on Synthesis of Virus-Induced Interferon in Cultures of Chick Embryonic Cells of Different Ages

Substance	Interferon titer (in $\log \text{PID}_{50}/0.5 \text{ ml} \pm \text{m}$)	
	one-day CES	five-day CES
None added	1.68 ± 0.07	2.89 ± 0.11
Adrenalin	1.61 ± 0.08	2.37 ± 0.14
P	>0.05	<0.05
None added	1.68 ± 0.07	2.89 ± 0.11
Theophylline	2.23 ± 0.10	2.50 ± 0.07
P	<0.01	<0.05
None added	1.87 ± 0.09	2.89 ± 0.11
Adrenalin + theophylline	1.81 ± 0.12	2.15 ± 0.06
P	>0.05	<0.01

Legend. PID_{50} - dose of interferon inhibiting plaque formation of vesicular stomatitis test virus by 50%.

In the same culture adrenalin did not affect interferon formation but reduced the level of interferon production when enhanced by theophylline to the control level (Table 1). Only after the second day of culture of the CES did adrenalin inhibit interferon synthesis (Fig. 3).

In the concentration used the preparations had no cytotoxic action on the cells.

The results could indicate that the action of adrenalin and theophylline on interferon production in the system used may be attributable to their influence on the intracellular cyclic AMP level. This is shown by the mutually potentiating inhibitory action of the two substances on interferon synthesis in the five-day CES. Adrenalin, which facilitates the formation of cyclic AMP, and theophylline which prevents its breakdown, are known to lead to the much greater accumulation of cyclic AMP within the cell when given together than when given separately [8].

they were given together (Table 1).

The substances had a marked action on interferon formation in 5-day CES even if added to the maintenance medium 4 h after infection of the cells. However, 6 h after the beginning of infection, the substances did not affect interferon production (Fig. 2). Preliminary treatment of the CES with adrenalin and theophylline in concentrations changing the level of interferon production for 4 h at 37°C did not affect the degree of adsorption of the interferonogen on the cells compared with the control. The substances did not affect the reproduction of vesicular stomatitis, Newcastle disease, and Chikungunya viruses in the CES or influenza B virus (Lee strain) in developing chick embryos.

The results are evidence that the probable point of application of action of the substances is interferon itself and not the initial stages of interaction between the interferonogen and the cell.

In a one-day CES theophylline not only did not reduce the yield of interferon, but increased it considerably. In

By contrast with the activity of phosphodiesterase (the enzyme decomposing cyclic AMP), the activity of adenylyl cyclase, which assists in cyclic AMP formation, and the cyclic AMP level itself were maximal in the "old" cultures, when the cells were in the resting phase with minimal growth potential. Adenylyl cyclase activity and the cyclic AMP level are much lower in growing cells [9, 12]. This could evidently explain the fact that theophylline affects interferon production in CES of all ages, but adrenalin does so only in older cells.

The action of cyclic AMP in the cell is based on its activation of cell protein kinases, as a result of which certain cell substrates, including histones, undergo phosphorylation [7]. This leads to derepression of the cell genome [7]. Presumably in "old" CES, where the intracellular cyclic AMP level is highest, theophylline and adrenalin in the presence of virus infection bring about the accumulation of cyclic AMP to an extent at which the cell genome is derepressed not only for interferon synthesis but also for synthesis of the repressor of interferon formation. A similar hypothesis was put forward by Dianzani et al. [6]. If this is so, synthesis of the repressor takes place in the early stages of infection of the cells, for the action of the preparations was seen to its greatest advantage during the first 4 h of action of infection (Fig. 2). Elevation of the cyclic AMP level in young cells by the action of theophylline can evidently reach only the level that facilitates interferon synthesis and not the level that facilitates synthesis of its repressor.

The results described above are indirect evidence of the possible role of endogenous cyclic AMP in the process of virus-induced interferon production in cell culture. The low level of cyclic AMP in "young" cells is possibly one cause of the reduced ability of these cells to form interferon [5].

LITERATURE CITED

1. F. N. Reizin and N. A. Zeitlenok, *Byull. Éksperim. Biol. i Med.*, No. 12, 65 (1969).
2. A. C. Allison, *Chem. Phys. Lipids*, 8, 374 (1972).
3. T. Bron and S. Rous, *Biochim. Biophys. Acta*, 237, 156 (1971).
4. R. W. Butcher and E. W. Sutherland, *J. Biol. Chem.*, 237, 1244 (1962).
5. D. Carver and P. Marcus, *Virology*, 32, 247 (1967).
6. F. Dianzani, P. Neri, and M. Zucca, *Proc. Soc. Exp. Biol. (New York)*, 140, 1375 (1972).
7. T. A. Langan, *Science*, 162, 579 (1968).
8. J. P. MacManus, J. F. Whitfield, and T. Youdale, *J. Cell. Physiol.*, 77, 103 (1971).
9. F. Marks and W. Grimm, *Nature New Biol.*, 240, 178 (1972).
10. S. E. Mayer, B. Williams, and J. M. Smith, *Ann. New York Acad. Sci.*, 139, 686 (1967).
11. G. A. Robison, R. W. Butcher, and E. W. Sutherland, *Ann. Rev. Biochem.*, 37, 149 (1968).
12. J. P. Schwartz, N. R. Morris, et al., *J. Biol. Chem.*, 248, 2699 (1973).