Regulation of 2′,5′-oligo(A) synthetase activity in theophylline-treated NIH 3T3 cells

A.V. Itkes, K.T. Turpaev, O.N. Kartasheva, V.L. Tunitskaya, C.A. Kafiani and E.S. Severin

Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow 117984, USSR

Received 14 November 1983

Sequential treatment of NIH 3T3 cells with theophylline and actinomycin D results in an enhancement of 2′,5′-oligo(A) synthetase activity 2–3-fold exceeding that induced by treatment with theophylline only. This 'superinduction' phenomenon suggests the existence of a negative control of the enzyme that involves a labile, transcription-dependent factor.

Theophylline *cAMP* *2′,5′-Oligo(A) synthetase* *Actinomycin D* *Superinduction*

1. INTRODUCTION

2′,5′-Oligo(A) synthetase is the enzyme which produces an important regulator of cell activity, 2′,5′-oligoadenylate, an oligonucleotide that consists of a few (usually 3 or 4) AMP residues connected to each other by 2′,5′-phosphodiester bonds. The main biochemical manner of 2′,5′-oligo(A) action involves activation of a specific latent endoribonuclease (RNse L, RNse F) which hydrolyses single stranded RNA [1]. 2′,5′-Oligo(A) synthetase activity is usually found in interferon-treated cells, and the antiviral and antiproliferative effects of interferon are mediated, at least partially, by a substantial elevation of 2′,5′-oligo(A) [1]. An enhancement of the enzyme activity was also observed during cell differentiation [2], regeneration [3], and decrease in the rate of cell proliferation [4]. 2′,5′-Oligo(A) is likely to be involved in the regulation of the above processes.

We have shown [5–7] that an elevation of 2′,5′-oligo(A) synthetase activity follows a rise in the intracellular cAMP level induced by different agents including theophylline, a specific inhibitor of the cAMP phosphodiesterase (PDE). We show here that an additional, 2–3-fold increase in enzyme activity is observed if actinomycin D is added to the culture, when the theophylline-dependent induction of 2′,5′-oligo(A) synthetase has begun. This procedure is referred to here as a sequential treatment with theophylline and actinomycin D.

2. METHODS

2.1. *Cell culture*

The NIH 3T3 cell line was cultivated in Eagle's medium supplemented with 10% RPMI 1640 medium and 10% fetal calf serum. Rapidly growing cells ($\geqslant$ 24 h before confluence) were used in our experiments.

The cells were treated with theophylline (2 mM) followed by actinomycin D (1 μg/ml) where indicated.

2.2. *2′,5′-Oligo(A) synthetase assay*

The cells were detached with Versen solution, collected by centrifugation and resuspended in 30 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10% glycerol, 0.05% Triton X-100, 20 mM Tris-HCl (pH 8.0). The cell suspension was incubated for 30 min at 4°C then centrifuged for 6 min at 8000 × *g*, the supernatant being used to obtain a protein fraction lacking 2′-phosphodiesterase activity, and the 2′,5′-oligo(A) synthetase was assayed as in [5].

2.3. *cAMP assay*

The cAMP level was assayed as in [5].

Published by Elsevier Science Publishers B.V.
001457593/84/$3.00 © 1984 Federation of European Biochemical Societies

3. RESULTS AND DISCUSSION

3.1. *cAMP level in the cells sequentially treated with theophylline and actinomycin D*

After theophylline addition to the culture medium, a transient 2.5-fold elevation of the intracellular cAMP level was observed. In about 8 h, cAMP decreased to the initial level and remained constant for at least 16 h (fig.1).

A second increase in the cAMP level was observed when the theophylline-containing medium was replaced (after 10 h incubation) by the actinomycin D-containing medium (fig.1). This second cAMP elevation could be due to inhibition of synthesis of mRNA for inducible cAMP PDE. It is known that cAMP PDE can be induced by high concentrations of cAMP [8]. In our case, the cells exposed to actinomycin D were pretreated with theophylline which induced cAMP enhancement. Hence cAMP-dependent induction of cAMP PDE might be expected. Actinomycin D inhibition of the synthesis of mRNA for labile inducible cAMP PDE would result in a fall in the total cAMP PDE activity and, consequently, in a rise in cAMP level.

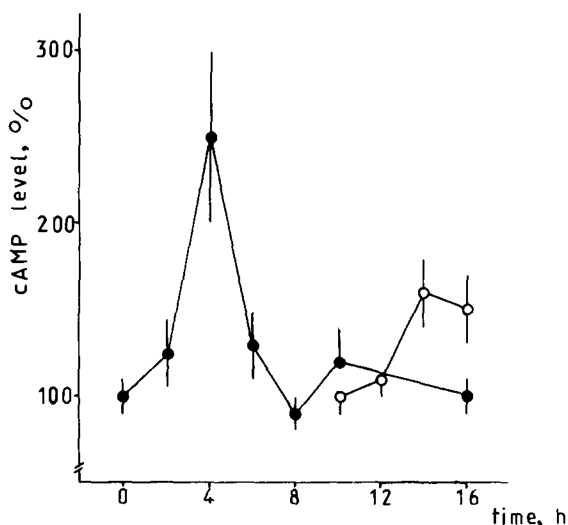

Fig. 1. cAMP level in NIH 3T3 cells treated with theophylline (●) or with theophylline and actinomycin D sequentially (○). The theophylline-containing medium was replaced by actinomycin D-containing medium 10 h after theophylline addition. The cAMP level in untreated cells remained at the initial level for at least 24 h. Values are means (±SE) of 4 independent measurements.

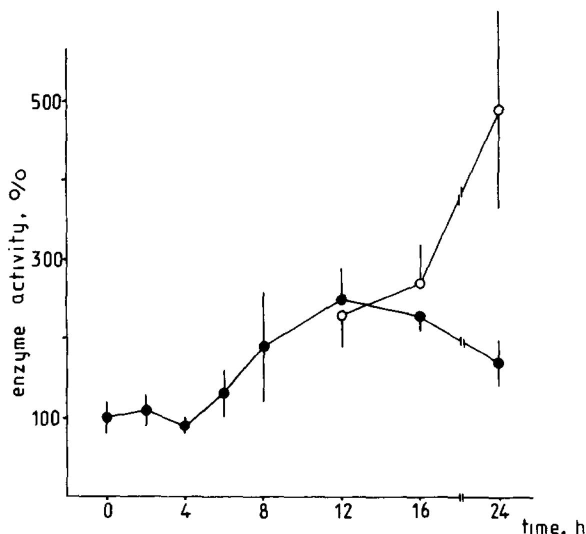

Fig. 2. 2′,5′-Oligo(A) synthetase activity in NIH 3T3 cells treated with theophylline (●) or with theophylline and actinomycin D sequentially (○). The theophylline-containing medium was replaced by actinomycin D-containing medium 10 h after theophylline addition. Enzyme activity is expressed as pmol AMP incorporated into the fraction of 2′,5′-oligo(A) trimer/1 h per mg protein of the lysate fraction lacking 2′-phosphodiesterase activity (see section 2). Enzyme activity in untreated cells and in cells treated with actinomycin D did not vary from the initial level more than 40% during at least 24 h. Values are means (±SE) of 4 independent measurements.

This is surely one of the possible explanations for the two-phase cAMP increases observed; direct experiments are necessary to test this proposal.

3.2. *2′,5′-Oligo(A) synthetase activity in the cells sequentially treated with theophylline and actinomycin D*

Treatment of cells with theophylline (or with other agents that cause cAMP elevation) results in 2′,5′-oligo(A) synthetase induction [5-7]. Fig. 2 demonstrates a 2.5-fold increase in the enzyme level caused by 2 mM theophylline (the concentration used in our experiments). The rise in enzyme activity began about 8 h after theophylline addition and had attained a maximal level by 12 h.

Sequential treatment of the cells with theophylline and actinomycin D (10 h incubation with theophylline and subsequent change of the medium and incubation with actinomycin D)

resulted in a more prolonged enhancement of the 2′,5′-oligo(A) synthetase activity; 24 h after theophylline addition (i.e. in 14 h after actinomycin D addition) the activity was 5-fold higher compared to the initial level (fig.2).

The rate of enzyme activity enhancement depended on the time of incubation of the cells with both agents; maximal activity was attained after 10 h incubation with theophylline followed by the change of the medium and incubation with actinomycin D for 14 h (table 1).

The second rise in cAMP level observed with actinomycin D could not induce 2′,5′-oligo(A) synthetase because actinomycin D prevents mRNA synthesis. Indeed, theophylline and actinomycin D, added simultaneously to the medium, caused a rise in cAMP level similar to that induced by theophylline only, but no enhancement of 2′,5′-oligo(A) synthetase activity occurred during at least 24 h (not shown). The cAMP-dependent induction of the enzyme caused by adrenaline treatment of the cells was also prevented by actinomycin D [7].

Nevertheless, the sequential (but not simultaneous) treatment of the cells with theophylline and actinomycin D resulted in a rise in the 2′,5′-oligo(A) synthetase level that was 2–3-fold higher than that caused by treatment with theophylline only (fig.2).

Table 1

2′,5′-Oligo(A) synthetase activity in NIH 3T3 cells sequentially treated with theophylline and actinomycin D

Time of incubation with theophylline (h)	Time of incubation with actinomycin D (h)	Relative activity of 2′,5′-oligo(A) synthetase
0	0	1.0 ± 0.2
0	24	0.9 ± 0.3
24	0	1.7 ± 0.3
16	8	2.8 ± 1.0
14	10	4.9 ± 1.5
12	12	4.1 ± 1.5

Cells were sequentially treated with theophylline and actinomycin D (see section 2); 2′,5′-oligo(A) synthetase was assayed 24 h after theophylline addition except in control where the assay was carried out with untreated cells (row 1) or actinomycin D-treated cells 24 h after the addition of the antibiotic (row 2). Values are means (± SE) of 4 independent experiments

These data suggest that at late stages of the theophylline-dependent induction of 2′,5′-oligo(A) synthetase, prevention of further enhancement of the enzyme level requires RNA synthesis. Inhibition of RNA synthesis by actinomycin D results in a more prolonged rise in the enzyme activity, the so-called 'superinduction'.

The molecular mechanism of this process is not clear, and further experiments are necessary to draw any conclusions. It should be noted, however, that similar 'superinduction' of 2′,5′-oligo(A) synthetase has been observed upon sequential treatment of chicken embryonic fibroblasts with interferon and cycloheximide [9]. The authors have supposed that the phenomenon involves a hypothetical 'labile protein agent' which inactivates mRNA for 2′,5′-oligo(A) synthetase: when synthesis of this labile protein was inhibited by cycloheximide, enzyme 'superinduction' occurred [9]. Our data agree with this hypothesis: in our experiments, the synthesis of the labile protein agent was supposedly inhibited by actinomycin D. In addition, these data suggest that the mechanisms of cellular regulation of interferon-induced and cAMP-induced 2′,5′-oligo(A) synthetase are similar.

REFERENCES

[1] Lengyel, P. (1981) in: Interferon 3 (Gresser, I., ed.) pp. 78–99, Academic Press, London.
[2] Friedman-Einat, M., Revel, M. and Kimchi, A. (1982) Mol. Cell. Biol. 2, 1472–1480.
[3] Etienne-Smekens, M., Goldstein, J., Ooms, H.A. and Dumont, J.E. (1983) Eur. J. Biochem. 130, 269–273.
[4] Krishnan, I. and Baglioni, C. (1981) Mol. Cell Biol. 1, 932–938.
[5] Itkes, A.V., Turpaev, K.T., Kartasheva, O.N., Vagonis, A.I., Kafiani, C.A. and Severin, E.S. (1982) Biochem. Int. 5, 15–21.
[6] Itkes, A.V., Krispin, T.I., Shloma, D.V., Balandin, I.G., Tunitskaya, V.L. and Severin, E.S. (1982) Biochem. Int. 5, 389–398.
[7] Itkes, A.V., Turpaev, K.T., Kartasheva, O.N., Kafiani, C.A. and Severin, E.S. (1983) Mol. Cell Biochem., in press.
[8] Rassel, T.K., Terasaki, W.L. and Appleman, M.M. (1973) J. Biol. Chem. 248, 1334–1340.
[9] Lab, M., Thang, M.N. and Soteriadou, K. (1982) Biochem. Biophys. Res. Comm., 105, 412–418.