The mechanisms of the cyclic AMP-dependent regulation of the enzymes of the 2′,5′-oligoadenylate system

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The cAMP-dependent induction of 2,5-oligoadenylate (2-5A) synthetase and cAMP-dependent inhibition of 2-5A phosphodiesterase are shown. Variations in activities of cAMP-dependent protein kinase and the enzymes of 2-5A metabolism in the cells deepening into the resting state were found to be compatible with the above finding. A scheme of coordinated action of cAMP and 2-5A is proposed.

Cyclic AMP *2′,5′-Oligoadenylate* *Cell regulation*

1. INTRODUCTION

Two regulatory biochemical systems, that of cyclic AMP (cAMP) and of 2′,5′-oligoadenylate (2-5A), are known to be involved in the control of cell growth and differentiation. The action of cAMP in eukaryotic cells is mediated by activation of a specific cAMP-dependent protein kinase. The kinase, in turn, phosphorylates substrate proteins, causing multiple biological effects [1]. 2-5A acts, at least partially, via activation of specific RNase (RNase L) which hydrolyzes single-stranded RNA that contributes to inhibition of protein synthesis and cell growth [2]. 2-5A was originally known as a mediator of interferon action. The level of this oligonucleotide rises in interferon-treated cells. Moreover, 2-5A mimics the antiviral and antiproliferative effects of interferon. Further studies suggested that the functions of 2-5A are not limited to mediation of interferon action; rather, 2-5A appears to be generally involved in regulation of cell proliferation.

Earlier it was shown that cAMP levels increased in interferon-treated cells [3]. However, there was no indication whether cAMP plays a role in the control of 2-5A metabolism.

The results of this study demonstrate the existence of interaction between the cAMP and 2-5A systems that allows us to propose a general scheme of their coordinated functioning in the regulation of cell proliferation.

2. METHODS

Cultivation of NIH 3T3 cells, assay of 2-5A synthetase, 2-5A phosphodiesterase, protein kinase, cAMP and 2-5A are detailed in [4–6].

3. RESULTS AND DISCUSSION

3.1. 2-5A synthetase activity increases in theophylline- or adrenaline-treated cells

The intracellular cAMP level was elevated by inhibition of phosphodiesterase of cAMP (cAMP PDE) with theophylline or by activation of adenylate cyclase with adrenaline.

Addition of 1 mM theophylline to the culture medium was followed by a transient rise in cAMP concentration in NIH 3T3 cells. Six h after theophylline addition, the cAMP content returned to the initial level and remained constant for at least 16 h. A transient increase in intracellular cAMP was also observed after addition of 1 μM adrenaline to the medium that declined to the initial level 6 h later (fig.1). Both increases in intracellular cAMP were not prevented by actinomycin D addition simultaneously with theophylline or adrenaline (not shown). However, if cells had been cultivated

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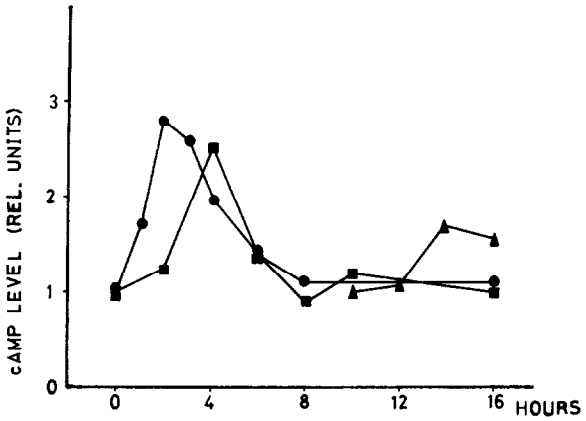

Fig.1. Level of cAMP in cells incubated with: adrenaline (●), theophylline (■), and 10 h with theophylline and then actimonycin D (▲).

for 10 h in the presence of theophylline, which caused an increase in cAMP, and then transferred to the medium with actinomycin D, a second increase in cAMP occurred (fig.1). This increase suggests the inhibition of synthesis of a short-lived form of cAMP PDE.

The cAMP elevation in cells treated with theophylline or adrenaline was followed by a rise in 2-5A synthetase activity. Fig.2 shows that by the 16th hour of cultivation of cells in the presence of 1 mM theophylline or 1 μM adrenaline, the enzyme activity increased 2–3-fold. The rise in 2-5A synthetase activity depended on the concentration of those agents in the culture medium (table 1). These

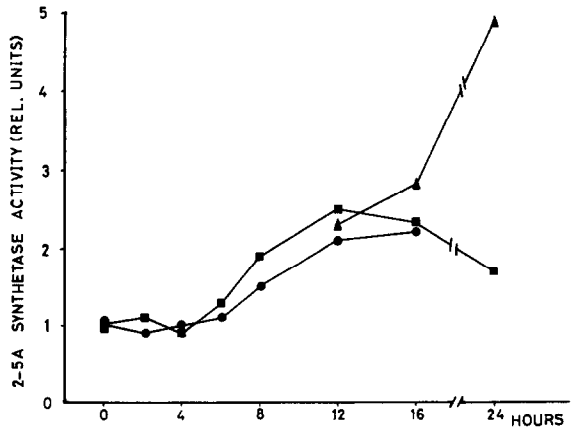

Fig.2. 2-5A synthetase activity in cells incubated with adrenaline (●), theophylline (■), and 10 h with theophylline and then actinomycin D (▲).

Table 1

2-5A synthetase activity in theophylline- and adrenaline-treated NIH 3T3 cells

Treatment	Reagent concentration (M)	2-5A synthetase activity (% of initial level)
Control	–	100 ± 20
Theophylline	10^{-4}	120 ± 20
Theophylline	10^{-3}	230 ± 20
Adrenaline	10^{-7}	140 ± 20
Adrenaline	10^{-6}	220 ± 50
Adrenaline	10^{-5}	240 ± 40
Adrenaline + 1 μg/ml actinomycin D	10^{-6}	90 ± 30

Enzyme activity was assayed 16 h after addition of theophylline or adrenaline to culture medium. Values are the mean (±SE) of 4 parallel experiments

results indicate that the rise in 2-5A synthetase level was caused by the increase in intracellular cAMP, independently of the manner in which it was achieved. The observed rise in 2-5A synthetase activity was prevented by actinomycin D addition simultaneously with one of the two agents used to increase intracellular cAMP (table 1). This suggests that the cAMP-dependent induction of 2-5A synthetase involves RNA synthesis.

A paradoxical effect was observed when actinomycin D was added 8 h after theophylline or later: the theophylline-induced rise in 2-5A synthetase activity in actinomycin D-treated cells exceeded that observed in untreated cells (fig.2). The strongest effect (5-fold increase in 2-5A synthetase activity) was observed when actinomycin D was added 10 h after theophylline.

As mentioned above, in cells treated with actinomycin D 10 h after theophylline addition a second transient rise in cAMP occurred. However, this rise could not contribute to the induction of 2-5A synthetase, since this additional induction would be blocked by actinomycin D present in the medium. Therefore, the stimulatory effect of actinomycin D can be qualified as superinduction, which involves a mechanism that does not require RNA synthesis.

3.2. 2'-PDE activity decreases in theophylline- or adrenaline-treated cells

In cells cultivated with 1 mM theophylline, 2-5A phosphodiesterase (2'-PDE) activity decreased to 40% of the initial level; a similar decrease was also observed in the presence of 1 μM adrenaline (fig.3). Thus, the cAMP rise in cells treated with either theophylline or adrenaline was followed by a rapid decline of 2'-PDE activity. However, it was not certain whether this effect was due to the rise in concentration of cAMP, since the time course of the decline was different with the two agents used. To clarify this point incubation of cells with adrenaline was carried out in the presence of 1 μM propranolol, which specifically blocks β-adrenergic receptors and prevents adenylate cyclase activation by adrenaline. Under these conditions, 2'-PDE activity remained unchanged, indicating that the adrenaline-dependent decrease in 2'-PDE activity was mediated by adenylate cyclase activation and an increase in cellular cAMP.

3.3. 2'-PDE activity decreases in the cell homogenate treated with protein kinase

To prove the concept that the cAMP effect on 2'-PDE is mediated by activation of cAMP-dependent protein kinase, NIH 3T3 cell homogenates were treated with purified catalytic subunit of cAMP-dependent protein kinase. The control homogenates lost 30% of their 2'-PDE activity during 8 h incubation, while in the presence of protein kinase, the homogenate lost 80% of the activity. Thus, phosphorylation of proteins of the cell homogenate produced the same effect on 2'-PDE activity as an increase in intracellular cAMP level, which is consistent with the above concept.

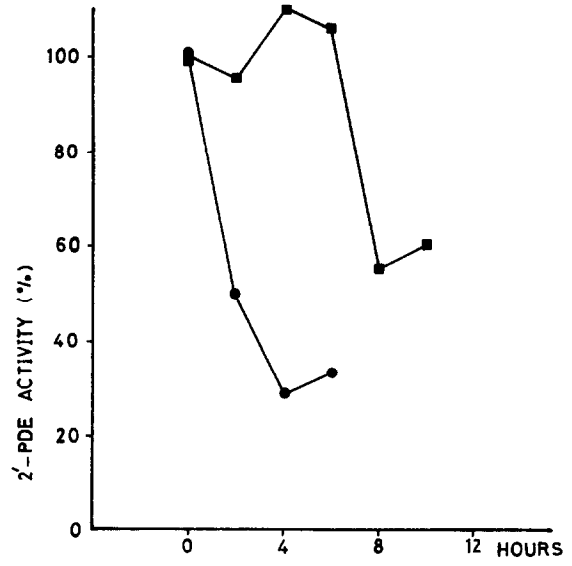

Fig.3. 2'-PDE activity in cells incubated with adrenaline (●) and theophylline (■).

3.4. Endogenous protein kinase activity increases in cells deepening into the resting state

To prove our hypothesis concerning cAMP-dependent regulation of the enzymes of 2-5A metabolism, variations of cAMP and 2-5A levels and activities of cAMP-dependent protein kinase, 2-5A synthetase and 2'-PDE in cells approaching the resting state were investigated.

To induce deepening into the resting state cells were cultivated in 0.5% serum medium that resulted in a rapid decrease in [^{3}H]thymidine incorporation. After 24 h, incorporation was 20%, and after 9 days 15% of the original level. cAMP concentration rose in these cells and reached a level of 135% at 48 h, decreasing thereafter (fig.4). Although the cAMP level varied slightly, the activity of cAMP-dependent protein kinase increase significantly in resting cells (fig.4). During 9 days of incubation, protein kinase activity increased nearly 7-fold.

At the same time, the activity of the thermostable protein inhibitor of cAMP-dependent protein kinase in resting cells decreased 3-fold com-

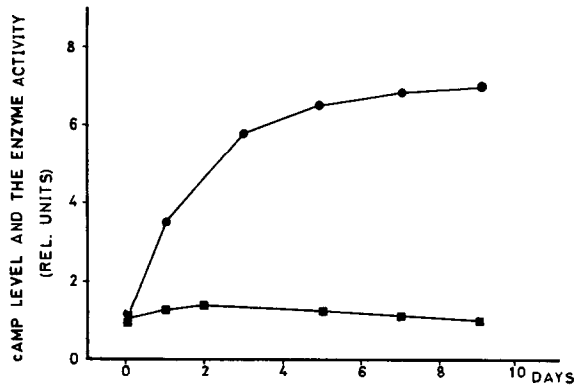

Fig.4. Level of cAMP (■) and activity of cAMP-dependent protein kinase (●) in cells deepening into the resting state.

pared with proliferating cells (not shown). This suggests that the observed increase in cAMP-dependent protein kinase activity is due to the decrease in the level of its specific protein inhibitor.

3.5. *Activity of 2-5A synthetase, 2'-PDE, and the level of 2-5A demonstrate coordinated behaviour in cells deepening into the resting state*

The activity of 2-5A synthetase increased continuously in cells approaching the resting state, increasing 8-fold during 9 days of observation (fig.5). The activity of 2'-PDE first fell during 5 days, and then rose again (fig.5). Intracellular content of 2-5A increased from $<0.3 \times 10^{-14}$ to $1-4 \times 10^{-14}$ mol per mg cell protein.

Thus, similar variations in the activities of 2-5A synthetase and 2'-PDE were observed in cells approaching the resting state in serum-deficient medium and in proliferating cells treated to elevate the cAMP level. The cAMP-dependent protein kinase activity increased in the cells deepening into the resting state. Hence, it is reasonable to suggest that cAMP-dependent protein kinase is involved in the regulation of activities of 2-5A synthetase and 2'-PDE in resting cells.

Our present results and the data of an earlier study [7] on 2-5A dependent activation of cAMP PDE are summarized in the scheme depicted in fig.6. Following this scheme, the interconnections between cAMP and 2-5A systems involve a negative feedback mechanism.

In addition, the data allow us to propose a novel explanation for the role of cAMP and 2-5A in the

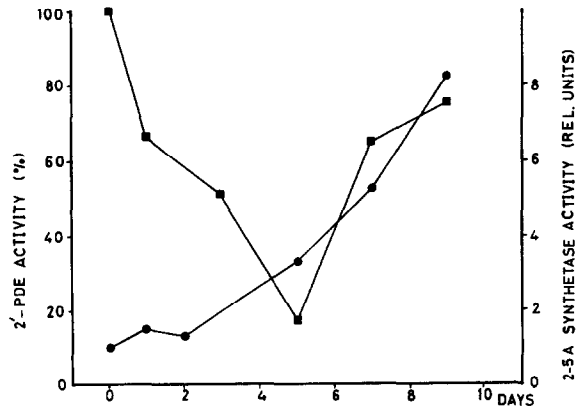

Fig.5. Activities of 2-5A synthetase (●) and 2'-PDE (■) in cells approaching the resting state.

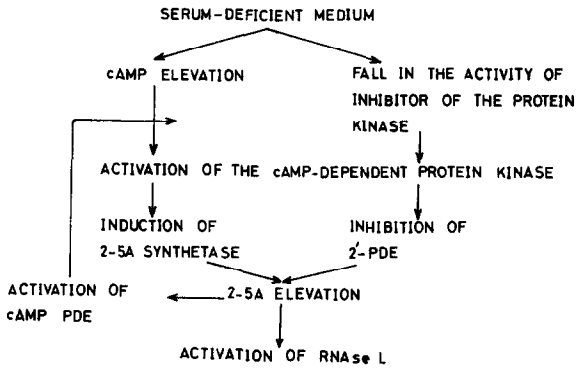

Fig.6. Scheme of interconnections between cAMP and 2-5A systems.

processes involved in deepening of cells into the resting state. It is well known that cAMP is an antiproliferative agent for a number of cell lines [8]. It is also known that the metabolism of resting cells is characterized by a high rate of RNA turnover and low rate of protein synthesis [9]. We believe that RNase L is involved in resting cell-specific metabolism. This idea agrees with a large body of experimental evidence for the activation of RNA hydrolysis and inhibition of protein synthesis by 2-5A [2].

Hence the hypothetical sequence of events resulting in the cell-specific alterations in RNA turnover and protein synthesis is as follows: cAMP elevation and/or fall in inhibitor activity causes activation of cAMP-dependent protein kinase; the kinase, in turn, is involved in the induction of 2-5A synthetase and in inhibition of 2'-PDE; as a result of these changes in the two enzyme activities, the 2-5A level rises, which leads to activation of RNase L. From this point of view, 2-5A is actually a messenger in an antiproliferative action of cAMP (or cAMP-dependent phosphorylation).

Finally, we wish to stress the point that the available experimental results obtained by other investigators are compatible with our hypothesis.

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