ACTION OF 3',5'-CYCLIC ADENOSINEMONOPHOSPHATE ON THE PROTEIN SYNTHESIZING CAPACITY OF THYROID POLYRIBOSOMES IN VITRO

Serge Lissitzky, Suzette Manté, Jean-Claude Attali and Guy Cartouzou

Laboratoire de Biochimie Médicale et Unité thyroïdienne de l'INSERM, Faculté de Médecine et de Pharmacie, Bd Jean Moulin, Marseille 5, France

Received March 10, 1969

The hypothesis of Sutherland et al. (1965) that cyclic 3',5'-adenosinemonophosphate (cAMP) might be the common intracellular mediator of the action of various hormones on their target tissue has received during the past few years increasing amount of evidences. Thyr oid-stimulating hormone (TSH) increases cAMP levels in thyroid slices (Gilman and Rall, 1966) and homogenates (Klainer et al., 1962). Conversely cAMP or its dibutyryl derivative mimics several TSH effects on the thyroid tissue such as the stimulation of phospholipid metabolism and glucose oxidation (Pastan and Macchia, 1967 ; Pastan, 1966), intracellular colloid droplet formation (Pastan and Wollman, 1967), radioiodine discharge (McKenzie, 1967) and iodide trapping (Wilson et al., 1968) in isolated thyroid cells.

The immediate intracellular target system of cAMP is still unknown. Although the initial experiments of Klainer et al. (1962) suggested a possible action of cAMP on the activation of phosphorylase, no definitive evidence that it might exert its regulatory activity as an effector of key (allosteric) enzymes or by another mechanism has yet been given (Robison et al., 1968). An alternative formulation of the action of cAMP is that it might be a regulator of messenger ribonucleic acid translation in polyribosomes. Such an effect should explain most of the early effects of TSH on the thyroid tissue.

It has been shown by Monroy et al. (1965) that mild tryptic digestion of ribosomes of unfertilized sea urchin eggs could promote their own protein synthesizing activity by unmasking of

polyribosomal mRNA. In this paper, similar experiments were carried out using thyroid polyribosomes and the effect of trypsin digestion was compared to that of cAMP addition. It is concluded that cAMP stimulates the protein synthesizing capacity of thyroid polyribosomes and ribosomes in vitro but likely in a different way than trypsin preincubation.

METHODS. Sheep thyroid polyribosomes have been isolated and tested for their protein synthesizing capacity in the presence of L-[^{14}C]leucine (100 µc/µmole) according to Cartouzou et al. (1967). Unless otherwise noted trypsin treatment was carried out by adding 30 µl of the protease solution at the adequate concentration in modified medium M of Munro et al. (1964) (containing 50 mM Mg^{++}) to 60 µl of polyribosome suspension (about 3 mg RNA/ml). After incubation for 10 min at 37°, 35 µl of a trypsin inhibitor solution was added to obtain a molar ratio of inhibitor to trypsin of 1.1 and a further 5 min incubation period at 37° was allowed to proceed. An aliquot of the mixture was used for the assay of amino acid incorporation. Preincubation in the presence of cAMP or other nucleotides was performed in the same manner with the omission of trypsin and trypsin inhibitor solutions and their replacement by 30 µl of nucleotide solution at the convenient concentration and 35 µl of medium M respectively. Rat liver and sheep thyroid ribosomes were prepared from the nuclei-free DOC-treated 20,000 g pellet of homogenates in 50 mM Tris pH 7.6, 25 mM KCl and 5 mM $MgCl_2$ according to Howell et al. (1964). All other methods have been described previously (Cartouzou et al., 1967). 2 x cryst. trypsin and cryst. pancreatic trypsin inhibitor were from Worthington (Freehold, USA), TSH (thytropar) from Armour (Kankakee, USA), cAMP and cUMP from Calbiochem (Los Angeles, USA).

RESULTS AND DISCUSSION. The preparations of sheep thyroid polyribosomes used in these experiments showed a [^{14}C]leucine-incorporating activity similar to that described previously (Cartouzou et al., 1967) and the same sedimentation profile in 10-30 % sucrose gradient. Although the specific protein synthesizing activity varied with different preparations of polyribosomes, liver cell sap was always shown to be more active than sheep thyroid cell sap and was used in subsequent experiments.

Fig. 1 shows the action of preincubation with increasing amounts of trypsin on the ability of polyribosomes to incorporate

[^{14}C]leucine into proteins. In the conditions used, a concentration of trypsin of 0.04 µg/ml provokes the maximum stimulating effect. With concentrations of 0.1 to 40 µg/ml a progressive

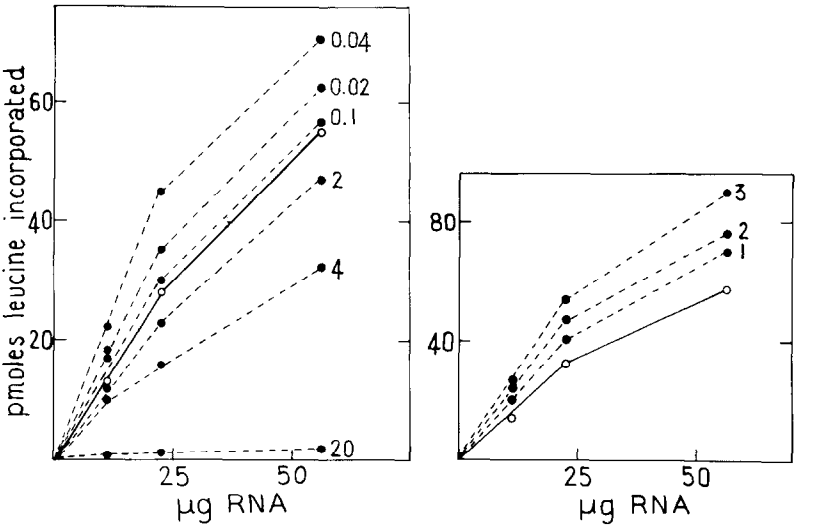

Fig. 1 (left). Effect of preincubation with trypsin on [^{14}C]leucine incorporation by thyroid polyribosomes. The protein-synthesizing activity was measured in the following conditions : for a final volume of 0.25 ml the concentration of the reaction mixture components were : Tris (pH 7.6) 20 mM, magnesium acetate 50 mM, NaCl 40 mM, KCl 100 mM, 2-mercaptoethanol 6 mM, L-[^{14}C]leucine (S.A. : 100 µc/µmole) 0.05 mM, ATP 5 mM, GTP 0.1 mM. Bovine serumalbumin was added to bring the protein content up to 2.2 mg in 0.25 ml. A cell sap protein to ribosomal RNA ratio of 30 was used. Incubation time 30 min at 37°. ○ , no trypsin treatment ; ● , 0.04, 0.02, 0.1, 2, 4 and 20 µg/ml trypsin.

Fig. 2 (right). Effect of preincubation with trypsin or cAMP on [^{14}C]leucine incorporation by thyroid polyribosomes. 0, no treatment ; 1, cAMP 0.2 mg/ml ; 2, cAMP 0.4 mg/ml ; 3, 0.04 µg/ml trypsin.

decrease of the protein synthesizing capacity of polyribosomes is observed which indicates that ribosomal structure is more or less disrupted.

The stimulation of protein synthesis by trypsin treatment is the higher with polyribosomal preparations which initially exhibit the lower protein synthesizing capacity (Table I). Treatment with low concentrations of trypsin allows a maximum activity of polyribosomes *in vitro* whatever are the factors (batch differences of

Table I

Stimulation of protein synthesis by trypsin-treated thyroid polyribosomes

exp. no.	Leucine-incorporating activity (mμmole/mg RNA)		% stimulation by trypsin treatment
	without trypsin treatment	with trypsin treatment	
1	128	200	56
2	200	200	0
3	72	204	183
4	132	188	34
5	56	172	207

thyroid glands, duration of storage at - 196°, contamination with soluble proteins etc...) which determine the level of the initial synthesizing activity. In the same conditions of preincubation, replacement of trypsin by cAMP has the same effect as low concentrations of trypsin (Fig. 2). An optimal effect is observed with a concentration of cAMP of 0.2 mg/ml (Table II). Adenylic nucleotides such as 5'-AMP or ADP, and bovine TSH are inactive (Table II). Whatever was the initial synthesizing activity of polyribosomes, cAMP always stimulated protein synthesis to variable degrees *in vitro* as was observed with preincubation in the presence of trypsin.

However the mechanisms of action of trypsin and cAMP on polyribosome synthesizing activity are likely different. When the maximum stimulating effect of trypsin is obtained, cAMP is still able to produce an additional stimulation of protein synthesis which is roughly of an order of magnitude equal to the sum of the effects provoked individually by these factors (Table II).

This interpretation is strengthened by the following observations 1. thyroid polyribosomal preparations the protein synthesizing capacities of which are not stimulated by preincubation in the presence of trypsin (see Table I, exp. no 2) responded to cAMP addition by an increased protein synthesis (40 to 60 %) 2. poly U-directed polyphenylalanine synthesis by a thyroid ribosomal cell-free system is stimulated (up to 130 %) by cAMP addition

Table II

Effect of cAMP, adenylic nucleotides and TSH on the protein synthesizing capacity of polyribosomes.

Addition	Leucine-incorporating activity (mμmole/mg RNA)	% stimulation (+) or inhibition (-)
none	66	-
trypsin (0.04 μg/ml)	112	+ 69
cAMP (50 μg/ml)	84	+ 27
cAMP (200 μg/ml)	104	+ 57
cAMP (400 μg/ml)	90	+ 36
trypsin (0.04 μg/ml) + cAMP (200 μg/ml)*	135	+ 104
5'AMP (200 or 400 μg/ml)	63	- 4
ADP (200 or 400 μg/ml)	66	0
TSH (0.05 to 0.01 IU)**	66	0
TSH (0.1 IU)**	41	- 37

*preincubation with trypsin for 30 min followed by trypsin inhibitor addition and a subsequent 30 min incubation in the presence of cAMP. Leucine-incorporating activity was compared with that of the same assays without cAMP addition during the second period of preincubation and without trypsin and cAMP. Kinetic experiments have shown that the effect of preincubation in the presence of trypsin was maximum at 30 min.

**number of international unit per assay (0.25 ml).

(Table III). cUMP, 5'-AMP and TSH are ineffective. A similar effect of cAMP is observed in a rat liver ribosomal cell-free system but not in the S_{30} *E. coli* acellular system of Nirenberg and Matthaei (1961).

It is therefore clear that cAMP stimulates *in vitro* protein synthesis using mammalian ribonucleoprotein particles. Its mechanism of action and its specificity towards animal systems are under investigation.

The inability of TSH to stimulate protein synthesis by thyroid polyribosomes agrees with the idea that cAMP might be the effective intracellular mediator of the hormone action.

No information is available on the capacity of cAMP to stimulate specifically or unspecifically polyribosomes. However

Table III

Effect of cAMP on poly U-directed polyphenylalanine synthesis by a thyroid ribosomal cell-free system

	[^{14}C]phenylalanine incorporated in TCA-insoluble material (cpm)	%stimulation
complete system	2,510	-
+ cAMP (100 µg/ml)	3,320	32
+ cAMP (200 µg/ml)	4,830	74
+ cAMP (400 µg/ml)	5,880	134
+ cAMP (800 µg/ml)	5,500	119
without poly U	80	-
+ TSH (0.01 to 0.1 IU per tube)	2, 480 to 1,660	0
+ cUMP (400 µg/ml)	1,970	0
+ 5'AMP (400 µg/ml)	2,520	0

For a final volume of 0.25 ml the complete system contained : Tris (pH 7.6) 50 mM, KCl 10 mM, $MgCl_2$ 15 mM, GTP 0.2 mM, ATP 1 mM, PEP 10 mM, PEPkinase 10 µg, 19 casaminoacids (except phenylalanine) 0.1 mM, L-[^{14}C]phenylalanine (S.A. : 138 µc/µmole) 0.6 µM, poly U 0.2 mg, rat liver cell sap 2 mg-protein, sheep thyroid ribosomes 45 µg.

it seems more likely that the response of polyribosomes to cAMP is unspecific and leads to a general increase of enzyme synthesis depending upon the amount and variety of mRNAs present in polyribosomes. Therefore, the nature of the response of thyroid polyribosomes to cAMP should depend upon the biochemical differenciation of the thyroid cell and the uniqueness of its enzymatic composition. This interpretation agrees with the conclusions of Grahame-Smith et al. (1967) that cAMP, in addition to mediating the effect of adrenocorticotropic hormone on steroidogenesis, might also be the intracellular mediator of the tropic effect of this hormone on the adrenal.

REFERENCES

Cartouzou, G., Greif, R., Depieds, R. and Lissitzky S., Biochim. Biophys. Acta, 138, 80 (1967).
Gilman, G.A. and Rall, T.W., Fed. Proc., 25, 617 (1966).
Grahame-Smith, D.G., Butcher, R.W., Ney, R.L. and Sutherland, E.W., J.Biol.Chem., 242, 5535 (1967).

Howell, R.R., Loeb, J.N. and Tomkins, G.M., Proc.Natl.Acad.Sci.U.S., 52, 1241 (1964).
Klainer, L.M., Chi, Y.M., Friedberg, S.L., Rall, T.W. and Sutherland, E.W., J.Biol.Chem., 237, 1239 (1962).
McKenzie, J.M., Recent Progress in Hor.Res., 23, 1 (1967).
Monroy, A., Maggio, R., and Rinaldi, A.M., Proc.Nat.Acad.Sci.U.S., 54, 107 (1965).
Munro, A.J., Jackson, R.J. and Korner, A., Biochem.J., 92, 289 (1964).
Nirenberg, M.W. and Matthaei, J.H., Proc. Natl.Acad.Sci.U.S., 47 1588 (1961).
Pastan, I., Biochem.Biophys.Res.Com., 25, 14 (1966).
Pastan, I. and Macchia, V., J.Biol.Chem., 242, 5757 (1967).
Pastan, I. and Wollman, S.H., J.Cell.Biol., 35, 262 (1967).
Robison, G.A., Butcher, R.W. and Sutherland, E.W., Ann.Rev. Biochem., 37, 149 (1968).
Sutherland, E.W., Oye, I., and Butcher, R.W., Recent Progress in Hor.Res., 21, 623 (1965).
Wilson, B., Raghupathy, E., Tonoue, T., and Tong, W., Endocrinology, 83, 877 (1968).