HORMONAL REGULATION OF THYROIDAL PROTEIN PHOSPHOKINASE ACTIVITIES

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1. Introduction

In thyroid glands, as in many other tissues, two types of protein phosphokinase activities have been described: cAMP-dependent and cAMP-independent enzymes [1–5]. cAMP-dependent enzymes have a central role in the molecular mechanism of hormonal regulation [6–8]. In the thyroid these enzymes are activated by TSH [9,10]. While the increase of cAMP-dependent activity after acute TSH stimulation has been described by several authors [9,10], little is known about the modifications of this activity in glands after prolonged stimulation or absence of TSH [11], and nothing is known about the TSH effect on the activities of cAMP-independent enzymes.

In the present work we have studied the activities of cAMP-dependent and cAMP-independent enzymes in cytosols from thyroid glands of animals treated either with methylthiouracil (hyperstimulated) or with thyroxine (hypostimulated rats).

We demonstrated that TSH regulates both types of protein phosphokinase activities. After separation of multiple species of enzymes by sucrose gradient ultracentrifugation, we observed that TSH acts by selectively stimulating the activities of only some cAMP-dependent and cAMP-independent enzymes.

2. Materials and methods

2.1. *Animals*

Hyperstimulated glands were obtained by treatment of male Wistar rats with methylthiouracil (MTU) (0.1% in drinking water for several weeks). Hypostimulated glands were obtained by treatment of animals with thyroxine (2.2 mg/l in drinking water for the same period).

2.2. *Preparation of cytosol fraction*

Glands from 2–5 animals were pooled, weighed and homogenized in an all-glass homogenizer in 15 or 30 volumes (w/v) of 50 mM Tris–HCl buffer, pH 7.4, containing 5 mM $MgCl_2$, 25 mM KCl, 6 mM mercaptoethanol (MSH) (Buffer A) to which 0.25 M sucrose was added. Homogenates were centrifuged in a Spinco Rotor 50 for 2 h at 150 000 × *g*. The supernatants (cytosols) were collected and the protein phosphokinase activities determined either immediately in the total soluble fraction or after fractionation by sucrose gradient ultracentrifugation.

2.3. *Fractionation of protein phosphokinase activities by sucrose gradient ultracentrifugation*

Soluble proteins (2–4 mg in 1 ml, final concentration) were layered on the top of a 12 ml linear sucrose gradient (11–22% sucrose in buffer A). The gradients were centrifuged in SW 40 Ti Rotor, 38 000 rpm, 0°C for 41 h. They were fractionated automatically into 0.3 ml fractions by a density gradient fractionator (ISCO). In each fraction of the gradient histone (± cAMP) and casein (–cAMP) kinase activities were evaluated. For the determination of sedimentation coefficients (S) or different kinase peaks three markers

were used: myoglobin (2 S), horse radish peroxidase (4 S) and glucose oxidase (7.93 S).

2.4. *Estimation of protein kinase activities*

Histone and casein kinase activities were evaluated as described previously [12]. The final concentration of [^{32}P]ATP was 10^{-3} M (specific activity 50–60 cpm/pmol) for the kinetic study of total soluble proteins and 10^{-4} M (spec. act. 50–60 cpm/pmol) for the evaluation of enzymatic activities in individual fractions of sucrose gradients. The final concentrations of histone and casein were 4 mg and 3 mg/ml respectively, and of cAMP 10^{-6} M. The incubations were carried out at 30°C for 5 min (for kinetic studies) or 10 min (for the sucrose gradient fractions). Under the above conditions the reactions were linear up to 10 min.

Histone and casein cAMP-independent activities were evaluated in the presence of the thermostable inhibitor of cAMP-dependent phosphokinases [13] in order to inhibit the activities of free catalytic subunits. Thermostable inhibitor was prepared and purified up to the Sephadex G-75 filtration step [14].

3. Results

3.1. *Quantitative evaluation of cAMP-dependent and cAMP-independent kinase activities*

Comparative analysis showed that in glands exposed to prolonged TSH action (MTU treated animals) the activities of both types of enzymes are significantly higher than in hypostimulated glands (thyroxine treated rats) (fig.1). The differences between stimulated and unstimulated glands are greater for casein than for histone kinase activity (ratio A/B).

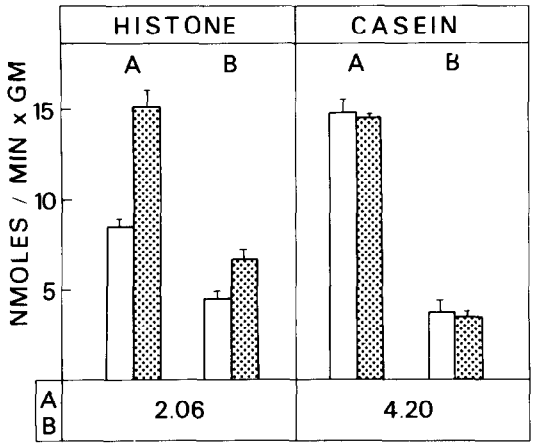


Fig.1. Quantitative evaluation of histone and casein kinase activities in cytosols from glands of animals treated with MTU (A) or thyroxine (B). Conditions of incubation were as described in Materials and methods. 20 μl aliquots of cytosol were used for the estimation of each enzyme activity. Empty columns; activities in the absence of cAMP, dotted columns: activities in the presence of cAMP. Mean values ± SEM of 4 separate experiments.

3.2. *Separation of different enzyme entities by sucrose gradient ultracentrifugation*

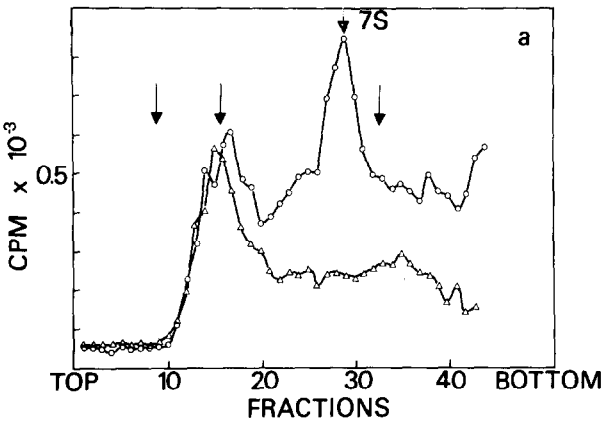
After centrifugation of cytosol proteins on sucrose gradients, cAMP-independent phosphokinase activities can be separated into two distinct peaks with sedimentation coefficients of 3.8–4.2 S and 7.0–7.5 S for casein kinase (fig.2a) and 3.5–3.7 S and 7.25–7.50 S for histone kinase activities (fig.2b). At present we cannot say if each one of the two peaks (about 4 S and about 7 S) represents two different enzymes (one phosphorylating casein and the other histone) or only one enzyme which phosphorylates the two substrates. Further analysis is necessary to decide between these two possibilities.

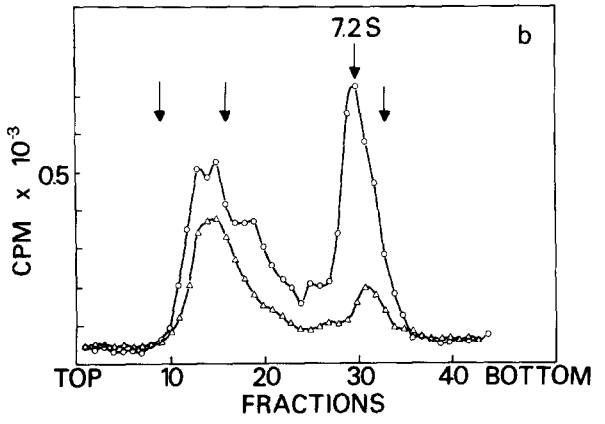
cAMP-dependent histone kinase activity was also separated into two peaks with sedimentation coefficients of 5.0 S and 6.3 S (fig.2c).

3.3. *TSH effect on different enzyme peaks*

Figures 2a–c show that sucrose gradient ultracentrifugation patterns of phosphokinase activities from stimulated and unstimulated glands are significantly different. Some kinase peaks are more sensitive to the hormonal influence than others. Among cAMP-independent enzymes the kinase peak of about 4 S is less sensitive to the hormonal modifications than 7 S peak. In hypostimulated glands the 7 S peak of casein kinase activity disappeared almost completely, while the same peak of histone kinase activity is dramatically reduced.

The patterns of cAMP dependent enzymes are also very different in the two types of glands. In the hypostimulated glands two distinct peaks of about the same activity are present (fig.2c). In hyperstimulated glands, however, only one of the two peaks is present (6.3 S), and is highly stimulated with respect to the





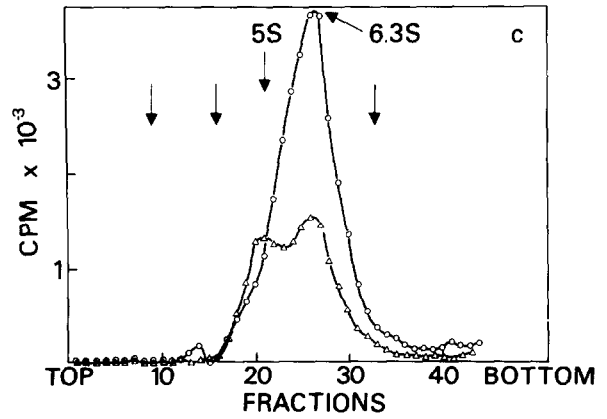


Fig.2. Sucrose gradient ultracentrifugation patterns of protein phosphokinase activities in cytosols from thyroids of MTU (o———o) and thyroxine (Δ———Δ) treated rats. For each type of gland the same amount of tissue was analyzed. The gradients were performed and fractionated as described in Materials and methods. 40 μl of each fraction were used for the estimation of each one of the following enzyme activities: (a) casein kinase; (b) cAMP-independent histone kinase and (c) cAMP-dependent histone kinase. Arrows indicate the positions of markers; from left to right: myoglobin (2 S), horse radish peroxidase (4 S) and glucose oxidase (7.93 S).

hypostimulated glands. The other peak (5 S) is almost completely absent.

These results clearly show that TSH selectively and preferentially regulates the activities of some protein phosphokinase activities.

4. Discussion

Our findings demonstrate that TSH regulates not only the cAMP-dependent but also the cAMP-independent protein phosphokinase activities. The question which can be raised is whether TSH controls the activities of the two types of enzymes by two different mechanisms, one involving the cAMP and another being independent on this nucleotide, or, does it act through the same mechanism? In the latter case TSH could stimulate the cAMP-independent kinase activity indirectly, by regulating the activity of cAMP-dependent histone kinase by a process similar to that of glucagon and adrenaline in the liver cell [6].

By the sucrose gradient ultracentrifugation method we separated the cAMP-dependent and cAMP-independent protein phosphokinase activities into several distinct peaks. It remains, however, to identify the nature of the intracellular proteins whose phosphorylation is catalyzed by each one of the peaks.

The sucrose gradient ultracentrifugation patterns of cytosol proteins, showed that the very important quantitative differences of protein phosphokinase activities between the two types of glands are not due to the proportional increase of all enzyme activities in the stimulated thyroids, but to the preferential modifications of some enzyme entities. Assuming that each peak catalyses the phosphorylation of a definite intracellular substrate, we can postulate that TSH regulates the phosphorylation of thyroidal proteins selectively.

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