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Activity of aminoacyl-tRNA synthetases in experimental hyperthyroidism in muscle tissues of the rabbit

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In cardial and femoral muscles of rabbits specific activities of aminoacyl-tRNA synthetases for twenty amino acids were generally similar, namely the activities towards amino acids and their amides, leucine, isoleucine, histidine, tyrosine, proline and serine were considerably lower than towards the remaining amino acids.

Specific activities of most aminoacyl-tRNA synthetases were higher in hyperthyroidism than in euthyreosis, and were higher in femoral muscle than in heart. The response to thyroxine treatment of individual aminoacyl-tRNA synthetases in both kinds of muscles varied with respect to most of the amino acids.

Thyroid hormones exert a significant influence on growth, development and homeostasis of the organism. Binding of thyroxine and triiodothyronine to specific receptors localized in the cell nucleus affect transcription of specific target genes [1–3]. The effect depends on the physiological state of the tissue [4, 5]. A selective increase in the total content of DNA and RNA in heart muscle has been evidenced [6].

Some tissues, such as liver and pituitary gland, contain a relatively large number of receptors, whereas testes and spleen, which exhibit a considerably weaker metabolic response to the thyroid hormones, contain fewer receptors [7].

Our previous experiments [8] have evidenced an increase of the acceptor activity of tRNA in thyroxinized rabbits, which might point to stimulation by thyroid hormones of either tRNA synthesis, or the activity of aminoacyl-tRNA synthetases.

The aim of the present study was to determine the activities of aminoacyl-tRNA synthetases in the heart and skeletal muscles in hyperthyroidic rabbits.

MATERIAL AND METHODS

The experiments were carried out on 12-week-old rabbits of mixed breed. In the experimental group (18 animals), hyperthyroidism was evoked by intramuscular administration of L-thyroxine (Sigma Chemical Co., St. Louis, U.S.A.) in a dose of 200 μg/kg body weight, during four consecutive days [9]. The level of triiodothyronine and thyroxine in serum was determined by the fluorescence polarization immunoassay method [10]. The heart and femoral muscles were taken from the thyroxinized and control rabbits.

Preparations of aminoacyl-tRNA synthetases were obtained from heart and femoral muscles.

The homogenized tissue was centrifuged at 5000 × *g* for 30 min, and microsomes were removed by ultracentrifugation at 105000 × *g* for 2 h. The enzyme protein was precipitated at 0.4-0.7 $(NH_4)_2SO_4$ saturation [11]. The precipitated fractions were dialysed against two changes of 0.05 M Tris/HCl buffer, pH 7.5, containing 5 mM KCl, 1 mM $MgCl_2$, 0.5 mM phenylmethylsulphonyl fluoride (PMSF) and 5% glycerol.

Protein content in the preparations was determined by the method of Bradford [12].

In the control experiment, the acceptor activity for amino acids of tRNA from rabbit liver was examined. This tRNA preparation was obtained from liver by phenol extraction according to Sein & Zubay [13, 14]. Then, it was fractionated by DEAE 52 column chromatography, and deaminoacylated [15]. tRNA Concentration was determined spectrophotometrically and expressed in absorbance units. The acceptor activity was determined by measuring binding of ^{14}C-labelled amino acids to tRNA. The assay system consisted, in a total volume of 250 µl, of: 100 mM Tris/HCl buffer, pH 7.5, 10 mM $MgCl_2$, 10 mM ATP, 10 mM KCl, 0.4 mM dithiothreitol (DTT), 0.1 mM PMSF, 3.0 A_{260} units of tRNA, 50 µg of enzymic protein, and 18.5 kBq ^{14}C-labelled amino acid. The incubation was carried out at 37°C for 20 min. From the incubation mixture, samples of 100 µl were withdrawn and transferred onto Whatman 3 MM discs, which were rinsed four times with cold trichloroacetic acid, and then by the Hokin solvent (0.8 ml 10 M KOH, 62.8 ml glacial acetic acid and ad 1 l 95% ethanol) and finally with ether. Radioactivity was measured after drying the discs, in an Intertechnique-France scintillation counter.

RESULTS

In the control group of rabbits the serum level of triiodothyronine was 0.82 ng/ml and that of thyroxine 1.92 µg/100 ml, whereas in the experimental, L-thyroxine-treated animals it was, respectively, 8 ng/ml and 22.3 µg/100 ml.

The activities of twenty aminoacyl-tRNA synthetases from heart and femoral muscles of thyroxinized rabbits, were compared to control tissues using tRNA from rabbit liver. Generally, the aminoacyl-tRNA synthetases fall into two groups: the enzymes with low activity, i.e. towards glutamic acid, aspartic acid, glutamine, histidine, isoleucine, tyrosine, serine, leucine, proline or asparagine, and those with rather high activity: towards alanine, phenylalanine, valine, arginine, glycine, cysteine, threonine or tryptophan (Fig. 1). The above differentiation was observed both in euthyreosis and hyperthyroidism but was not found in a homologous system in which both the enzyme and tRNA were from rabbit liver. Moreover the differentiation was more pronounced in hyperthyroidism than in euthyreosis, and more in the femoral than in heart muscle.

The response to thyroxine of individual aminoacyl-tRNA synthetases from the two kinds of muscle varied with respect to the majority of amino acids (Table 1). It was increased 1.7–5.4-

Table 1

The ratios of specific activity of aminoacyl-tRNA synthetases in hyperthyroidism and euthyreosis in rabbits.

The values were calculated from six experiments.

Amino acid	Muscles	
	Heart	Femoral
Alanine	3.6	5.2
Phenylalanine	0.9	2.1
Valine	1.2	2.2
Arginine	1.6	6.4
Lysine	2.3	4.5
Histidine	1.0	1.9
Tyrosine	0.6	1.7
Serine	0.9	5.9
Proline	2.3	3.5
Aspartic acid	0.6	3.1
Glycine	0.4	1.6
Cysteine	2.7	3.5
Asparagine	0.3	0.9
Glutamine	0.3	1.3
Threonine	0.4	0.8
Glutamic acid	1.1	1.6
Isoleucine	0.6	0.8
Tryptophan	1.7	1.2
Methionine	5.3	0.5
Leucine	5.8	1.7

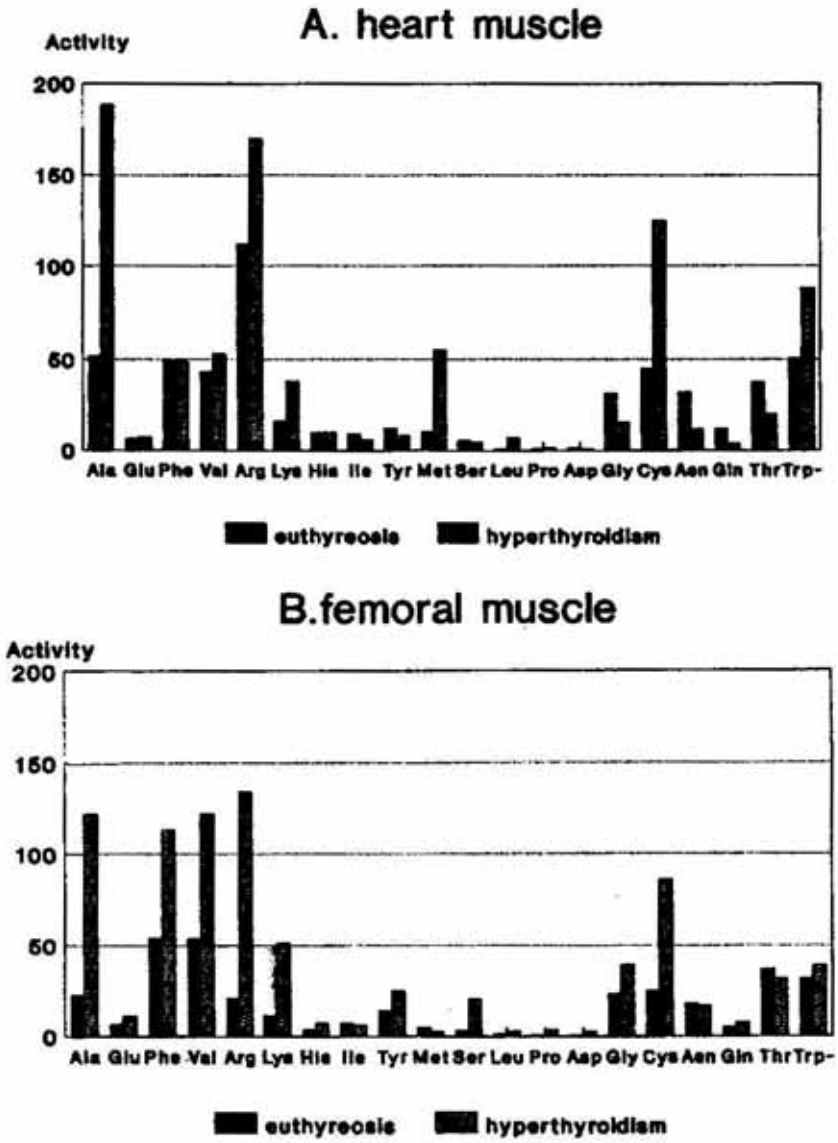

Fig. 1. Activity of aminoacyl-tRNA synthetases: A, in heart muscle, and B, in femoral muscle of thyroxine-treated ▨ and control rabbits ■.
The activity is expresses in picomoles of ^{14}C-labelled amino acid incorporated per 1 mg protein. The results are mean values from six experiments.

fold in heart muscle and 1.4–5.9-fold in femoral muscle. Also it should be stressed that large differences were observed in the response of individual synthetases to thyroxine treatment in both muscles, e.g. thyroxine treatment did not change the activity towards serine in heart but led to its about 6-fold increase in the femoral muscle. On the other hand, the activity towards methionine was increased only in heart.

DISCUSSION

Skeletal and cardiac muscles constitute two different types of striated muscle, although in both the contractile apparatus is closely similar [16].

The previous study [8] showed an increased acceptor activity of tRNA for five amino acids, induced by experimental hyperthyroidism in rabbits.

In the present study we have proved that this increase is due to the increased enzymatic activity in hyperthyroidism in skeletal and cardiac muscles of rabbits. An up-regulation of V-1 myosin expression in the cardiac ventricle of rabbit is well known [17], and the regulation of the same myosin isoform by thyroid hormones depends on the kind of target muscle [18].

Similarly, we have demonstrated differences in the response of cardiac and skeletal muscle to thyroxine.

Most of the studies performed so far dealt with the influence of thyroid hormones on the synthesis of mRNA associated with stimulation of nucleus receptors [18–20], but there are no data available concerning the effect of thyroxine on mRNA of aminoacyl synthetase. Tissues and organs of animals differ in the content of aminoacyl-tRNA synthetases. This might be related, among others, to the rate of cell growth and metabolic activity [21]. In the tissues with low mitotic coefficient, e.g. heart, the activity of aminoacyl-tRNA synthetases is lower than in other tissues [22].

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