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Seasonal differences in activity of rabbit liver tRNA and aminoacyl-tRNA synthetases specific for isoleucine and threonine under myocardial ischemia

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Activity of tRNA and aminoacyl-tRNA synthetases (AA-tRNA synthetases) in postribosomal supernatants from normal (control) rabbit liver and under 6, 12 and 24 h experimental myocardial ischemia (EMI) in autumn (September and October) and winter (December and January) have been compared. The results showed that acceptor activity of total tRNA for isoleucine and threonine under 6, 12 and 24 h EMI in autumn was higher by 18–29% than in winter. No differences were observed in acceptor activity of tRNA between normal groups of both seasons. The results of a study of AA-tRNA synthetase activities showed that the specific activity of isoleucyl- and threonyl-tRNA synthetases in liver under 6, 12 and 24 h EMI in autumn was higher by 17–37% than in winter. No differences in activity of aminoacyl-tRNA synthetases between normal groups of both seasons were observed. A decrease of tRNA acceptor activity under EMI in both seasons correlated with an increase of corresponding AA-tRNA synthetase activity which may be part of the compensatory mechanism of the cell to keep the normal range of protein synthesis under extreme conditions.

Key words: tRNA, aminoacyl-tRNA synthetases, protein synthesis, rabbit liver, seasons, myocardial ischemia

INTRODUCTION

Under myocardial ischemia, hypoproteinemia and hypoalbuminemia originate. Most of blood plasma and other proteins are synthesizing in the liver. An essential protein of blood plasma is albumin which is synthesizing only in the liver. Translation, the process of mRNA-encoded protein synthesis, requires a complex apparatus composed of the ribosome, tRNAs and additional protein factors, including aminoacyl-tRNA synthetases. Aminoacyl-tRNA formation is a key step in protein synthesis. This reaction is catalyzed by specific for each amino acid enzymes, aminoacyl-tRNA synthetases [1, 2], which catalyze the covalent attachment of an amino acid to its cognate transfer RNA [3, 4]. It is known that under myocardial ischemia protein synthesis is altered in the heart [5] and other organs, particularly in the liver [6, 7].

Our previous studies have shown that acceptor activity of rabbit liver tRNA for some amino acids decreased after 6, 12, 24 h EMI and reached the control level within 72 h, while the activities of the corresponding aminoacyl-tRNA synthetases of the liver were increasing [8–10]. Investigations confirm that the intensity of protein synthesis [11–13], gene expression [14, 15], acceptor activity of tRNA [16] and changes in ultrastructure of hepatocytes [13] depend on seasons of the year.

The objective of the present study was to examine the acceptor activity of tRNA for isoleucyne and threonine and the activity of the corresponding AA-tRNA synthetases of a normal rabbit liver and 6, 12 and 24 h after EMI in different seasons of the year. These amino acids were chosen because they are fully essential and their day requirement value and amount in proteins are less than of most of the other amino acids.

MATERIALS AND METHODS

Male rabbits (2.5–3.5 kg) were used. Control rabbits and rabbits after 6, 12 and 24 h EMI were anaesthetized and terminated according to the rules defined by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (License No 0028).

Acute myocardial ischemia was induced by occlusion of the left anterior descending coronary artery according to [17].

tRNA and aminoacyl-tRNA synthetases were isolated from normal (control) rabbit liver and 6, 12 and

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24 h after the beginning of EMI. These periods were chosen because essential alterations in protein synthesis intensity and level [8] as well as in the activity of rabbit liver tRNA, aminoacyl-tRNA synthetases were observed at these time points [9, 10].

Total tRNA was isolated from rabbit liver according to the Brungraber method (18) with subsequent deacylation as described earlier [19]. Isolation of total aminoacyl-tRNA synthetases and determination of their concentration were performed as in [20].

The acceptor activity of total tRNA for particular ^{14}C-labelled amino acids was determined as described in [21]. Quantitative determination of radioactivity of product bands was performed by liquid with the aid of a Delta-300 scintillation counter (count efficiency 60%). Activity of aminoacyl-tRNA synthetases was measured according to the initial rate of tRNA aminoacylation reaction with ^{14}C-labelled amino acids. The composition of a standard reaction mixture and the procedure were reported in [22]. The reliability of the data was estimated according to the Student distribution coefficient (t). Changes are statistically significant when $p < 0.05$.

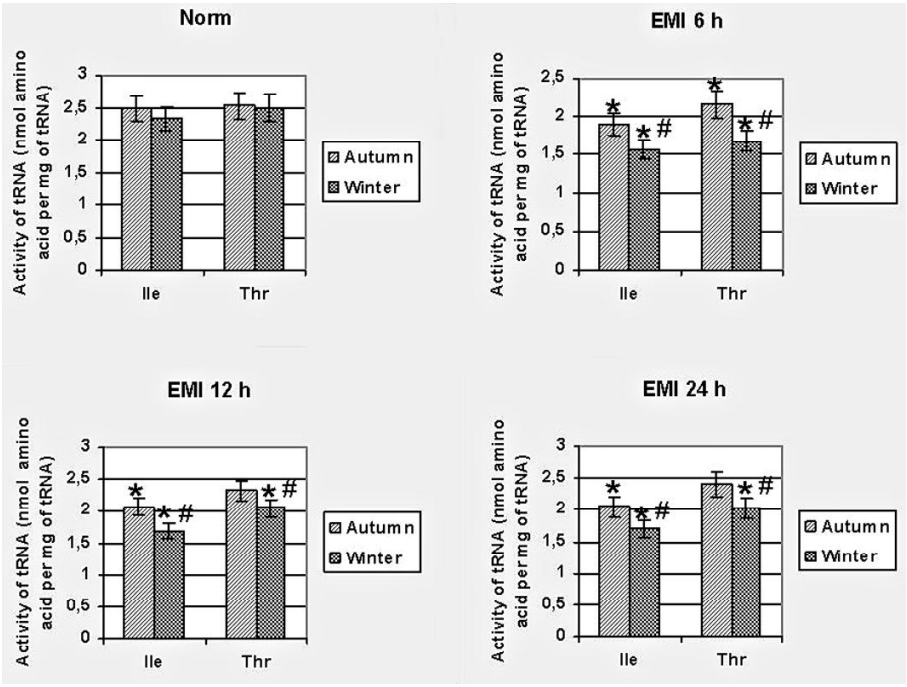

Fig. 1. Acceptor activities of specific tRNA of rabbit liver in norm and under 6, 12 and 24 h EMI in autumn and winter. Data represent results of 8–12 separate experiments; * – differences between control and experimental groups are statistically significant, # – differences between autumn and winter groups are statistically significant

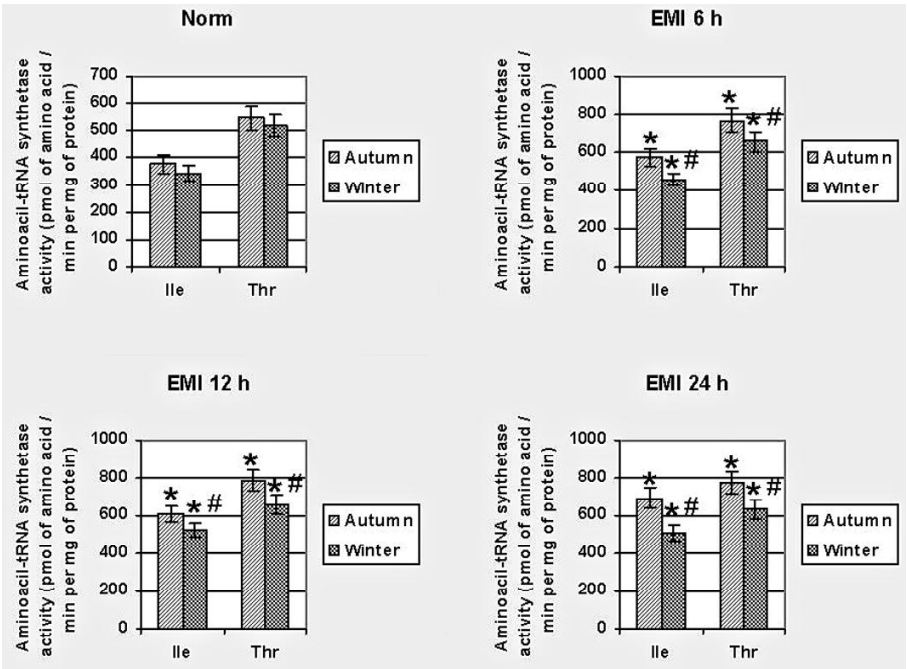

Fig. 2. Activities of specific aminoacyl-tRNA synthetases of rabbit liver in norm and under 6, 12 ir 24 h EMI in autumn and winter

Data represent results of 8–12 separate experiments; * – differences between control and experimental groups are statistically significant, # – differences between autumn and winter groups are statistically significant.

RESULTS AND DISCUSSION

Activity of tRNA and aminoacyl-tRNA synthetases of normal rabbit liver and under 6, 12 and 24 h EMI in autumn (September and October) and in winter (December and January) was compared. The results showed that acceptor activity of total tRNA for isoleucine and threonine under 6 h EMI in autumn was higher by 17–23%, under 12 h EMI by 19–22%, and under 24 h EMI by 18–20% than in winter (Fig. 1). No differences were observed in acceptor activity of tRNA between normal groups of both seasons.

As reported earlier [8, 19, 23], a decrease of acceptor activity of tRNA under EMI may be associated with formation of inactive molecules due to conformational changes of some tRNA molecules and is not related with losing the terminal CCA nucleotide triplet of the 3'-acceptor steam of these molecules.

In winter, acceptor activity of tRNA for isoleucine and threonine under 6, 12 and 24 h EMI decreased by 19–33% as compared to norm. In autumn, a statistically significant decrease (by 17–23%) was determined for isoleucine under 6, 12 and 24 h EMI and for threonine (by 17%) only under 6 h EMI as compared to norm. No differences were observed in acceptor activity of tRNA for threonine under 12 and 24 h EMI in autumn as compared to control.

Alterations of the acceptor activity of tRNA under EMI in different seasons may be associated with appearance of inactive tRNA conformers, as was shown for some tRNA under EMI [8, 24], and with alterations in activity of total tRNA methyltransferases which can cause differences in the step of methylation of some nucleotides of tRNA as described for total methyltransferase activity under 12 h EMI [25].

Results of the study of AA-tRNA synthetase activity of rabbit liver postribosomal supernatant showed that the specific activity of isoleucyl- and threonyl-tRNA synthetases under 6, 12 and 24 h EMI in autumn was higher than in winter (Fig. 2). No seasonal differences in activity of isoleucyl- and threonyl-tRNA synthetases of normal groups were observed.

In winter, the activity of isoleucyl- and threonyl-tRNA synthetases under 6, 12 and 24 h EMI increased by 23–51% and in autumn by 16% only after 12 h EMI, and the activity of arginyl-tRNA synthetase increased by 41–84% as compared to control.

Differences of the AA-tRNA synthetase activities in rabbit liver postribosomal supernatant under EMI in different seasons may be associated with an increased activity of inorganic pyrophosphatase which regulates aminoacyl-tRNA synthetase activity by cleavage of inorganic pyrophosphate, as shown earlier for 12 h EMI [25], and with alterations in the distribution of aminoacyl-tRNA synthetase activity between high molecular complexes and fractions of lower molecular complexes and free enzymes as reported under EMI [6] and other conditions [26]. Activity of aminoacyl-tRNA sinthetase may be regulated by phosphorylation / dephosphorylation as shown in [27]. There are no differences in autumn and winter activity of tRNA and AA-tRNA synthetase prepared from the liver of control group animals. Both components of trans-lation machinery showed lower changes in activity under 6, 12 and 24 h EMI in autumn than in winter. It may be related to a better common state of the organism's health and its ability to respond to different stress conditions and diseases such as myocardial ischemia and others. This response depends on the natural light period of the day, differences in the feeding of laboratory animals and the amount of some hormones, such as testosterone, estradiol, prolactin, gonadotropin, leptin, etc., excretion of which depends on seasons as has been noted for other subjects [15, 28–30]. The amount of some hormones, for example, epinephrine, changed under different stress conditions and ischemia. The decrease of acceptor activity of tRNA under EMI in both seasons is associated with the increase of the corresponding AA-tRNA synthetase activity, which may be part of the compensatory mechanism of the cell to keep protein synthesis in a normal range under extreme conditions.

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TRIUŠIŲ KEPENŲ tRNR IR AMINOACIL-tRNR-SINTETAZIŲ, SPECIFINIŲ IZOLEUCINUI IR TREONINUI, AKTYVUMAS MIOKARDO ISCHEMIJOS METU SKIRTINGU METŲ LAIKU

S a n t r a u k a

Palygintas tRNR ir aminoacil-tRNR-sintetazių (AA-tRNR-sintetazių) aktyvumas postribosominiame supernatante, kuris išskirtas iš triušių kepenų esant normai (kontrolė) ir praėjus 6, 12 ir 24 val. po eksperimentinės miokardo ischemijos (EMI) rudenį (rugsėjo–spalio mėnesį) ir žiemą (gruodžio–sausio mėnesį). Nustatyta, kad rudenį po 6, 12 ir 24 val. EMI tRNR gebėjimas akceptuoti izoleuciną ir treoniną yra 18–29% didesnis negu žiemą. Kontrolinių grupių triušių kepenų tRNR akceptinio aktyvumo skirtumų skirtingu metų laiku nerasta. AA-tRNR-sintetazių aktyvumo tyrimai rodo, kad izoleucil- ir treonil-tRNR-sintetazių aktyvumas po 6, 12 ir 24 val. EMI rudenį yra 17–37% didesnis negu žiemą. Nustatyta, kad kontrolinių triušių kepenų AA-tRNR-sintetazių aktyvumas rudenį ir žiemą nesiskyrė. Ir rudenį, ir žiemą tRNR akceptinio aktyvumo sumažėjimas EMI metu yra susijęs su atitinkamų specifinių AA-tRNR-sintetazių aktyvumo padidėjimu. Manoma, kad AA-tRNR-sintetazių aktyvumas padidėja kaip kompensacinis atsakas sumažėjus tRNR akceptiniam aktyvumui EMI metu tiek rudenį, tiek žiemą.