

EKSPERIMENTINIAI TYRIMAI

Seasonal differences in activities of rabbit liver tRNA and aminoacyl-tRNA synthetases specific for valine and arginine under myocardial ischemia

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

Summary. The objective of this study was to examine the acceptor activities of tRNA for amino acids, valine and arginine, and the activities of the corresponding aminoacyl-tRNA synthetases of normal rabbit liver and 6, 12 and 24 h after experimental myocardial ischemia in different seasons of the year.

Material and methods. Male rabbits (2.5–3.5 kg) were used. Acute myocardial ischemia was induced by occlusion of the left anterior descending coronary artery. tRNA and aminoacyl-tRNA synthetases were isolated from control rabbit liver and 6, 12 and 24 h after experimental myocardial ischemia in autumn (September and October) and winter (December and January). The acceptor activity of tRNA and the activity of valyl- and arginyl-tRNA synthetases were determined using ^{14}C -labeled amino acids, valine and arginine.

Results. The results showed that acceptor activity of rabbit liver tRNA for valine and arginine under 6, 12 and 24 h experimental myocardial ischemia in autumn was higher by 24–35% than in winter. Activities of rabbit liver valyl- and arginyl-tRNA synthetases under 6, 12 and 24 h experimental myocardial ischemia in autumn were lower by 15–32% than in winter. No differences in the activity of tRNA and aminoacyl-tRNA synthetases between control groups of both seasons were observed.

Conclusions. The experimental data suggest that acceptor activity of rabbit liver tRNA for valine and arginine and activity of valyl- and arginyl-tRNA synthetases under 6, 12 and 24 h experimental myocardial ischemia are different in autumn and winter. The decrease of acceptor activity of tRNA for valine and arginine after experimental myocardial ischemia correlates with an increase in the activity of valyl- and arginyl-tRNA synthetases both in autumn and winter. It may be part of the compensatory mechanism of the cell to keep synthesis of protein in a normal range under extreme conditions.

Introduction

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 aminoacyl-tRNA synthetases, enzymes specific for each amino acid (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 heart (5) and other organs, particularly in liver (6, 7).

Our previous studies have shown that acceptor activity of rabbit liver tRNA for some amino acids decreased at 6, 12, 24 h after experimental myocardial ischemia (EMI), and reached the control level within 72 h, while the activities of the cognate aminoacyl-tRNA synthetases of the liver increased at the same time (8–10). Performed investigations confirm that 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 this study was to examine the

acceptor activities of tRNA for valine and arginine and the activities of the corresponding aminoacyl-tRNA synthetases of normal rabbit liver and 6, 12 and 24 h after EMI in different seasons of the year.

Material and methods

Male rabbits (2.5–3.5 kg) were used. Control rabbits and rabbits under 6, 12 and 24 h EMI were anesthetized and killed 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 A. Toleikis *et al.* (17).

tRNA and aminoacyl-tRNA synthetases were isolated from control rabbit liver and at 6, 12 and 24 h after induction of EMI. These periods were chosen because essential alterations in the intensity and level of protein synthesis (8) as well as in the activity of rabbit liver tRNA, aminoacyl-tRNA synthetases were observed (9, 10).

Total tRNA was isolated from rabbit liver according to E. F. 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 -labeled amino acids was determined as described in (21). Quantitative determination of radioactivity in product bands was performed by liquid scintillation counter “Delta-300” (efficiency of counting – 60%). Activities of aminoacyl-tRNA synthetases were measured by the initial rate of tRNA aminoacylation reaction with ^{14}C -labeled amino acids. The composition of standard reaction mixture and the procedure were reported previously (22). Significance of the data was estimated by Student’s distribution coefficient (*t*). Differences are statistically significant when $p < 0.05$.

Results

The activities of tRNA and aminoacyl-tRNA synthetases of normal rabbit liver (control group) and under 6, 12 and 24 h EMI in autumn (September and October) and in winter (December and January) were compared. The results showed that acceptor activity of total tRNA for valine and arginine under 6 h EMI in autumn was higher by 24–35%, under 12 h EMI – by 30–32%, and under 24 h EMI – by 26% than in winter (Fig. 1). No differences were observed in acceptor activity of tRNA between control groups of both seasons.

In winter, acceptor activity of tRNA for valine and

arginine under 6, 12 and 24 h EMI decreased by 25–39% as compared to control. In autumn, a statistically significant decrease (by 16–26%) was determined for valine and arginine only under 12 and 24 h EMI as compared to control. No differences were observed in acceptor activity of tRNA for valine and arginine under 6 h EMI in autumn as compared to control.

Results of the study of aminoacyl-tRNA synthetase activity of rabbit liver showed that the specific activity of valyl- and arginyl-tRNA synthetases under 6, 12 and 24 h EMI in autumn was lower by 15–32% than in winter (Fig. 2). No seasonal differences in the activity of valyl- and arginyl-tRNA synthetases of control groups were observed.

In winter, the activity of valyl- and arginyl-tRNA synthetases under 6, 12 and 24 h EMI increased by 30–81% as compared to control. In autumn, the activity of valyl-tRNA synthetase increased by 16% only after 12 h of EMI, and the activity of arginyl-tRNA synthetase increased by 17–46% at all durations of EMI as compared to control.

Discussion

Obtained data indicate that under 6, 12 and 24 h EMI the acceptor activity of rabbit liver tRNA for valine and arginine in autumn was higher than in winter. Acceptor activities of tRNA for valine and arginine after EMI in winter were lower at all durations of EMI as compared to control. In autumn the activity of tRNA for valine and arginine was lower only under 12 and 24 h EMI, and remained without changes under 6 h EMI as compared to control. As reported earlier (8, 23) a decrease in the acceptor activity of tRNA under EMI may be associated with formation of inactive molecules due to conformational changes of some molecules of tRNA and is not related to the loosening of terminal CCA nucleotide triplet of the 3'-acceptor stem of these molecules. Alterations of the acceptor activity of tRNA after EMI in different seasons may be associated with the appearance of inactive tRNA conformers, as it was shown for some tRNA after EMI (8, 24) and with alterations in the 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).

The data on the activity of valyl- and arginyl-tRNA synthetases showed that the activity of these enzymes under EMI in winter was higher than in autumn. Activities of valyl- and arginyl-tRNA synthetases under EMI in winter are higher as compared to the control group. Activity of valyl-tRNA synthetase in

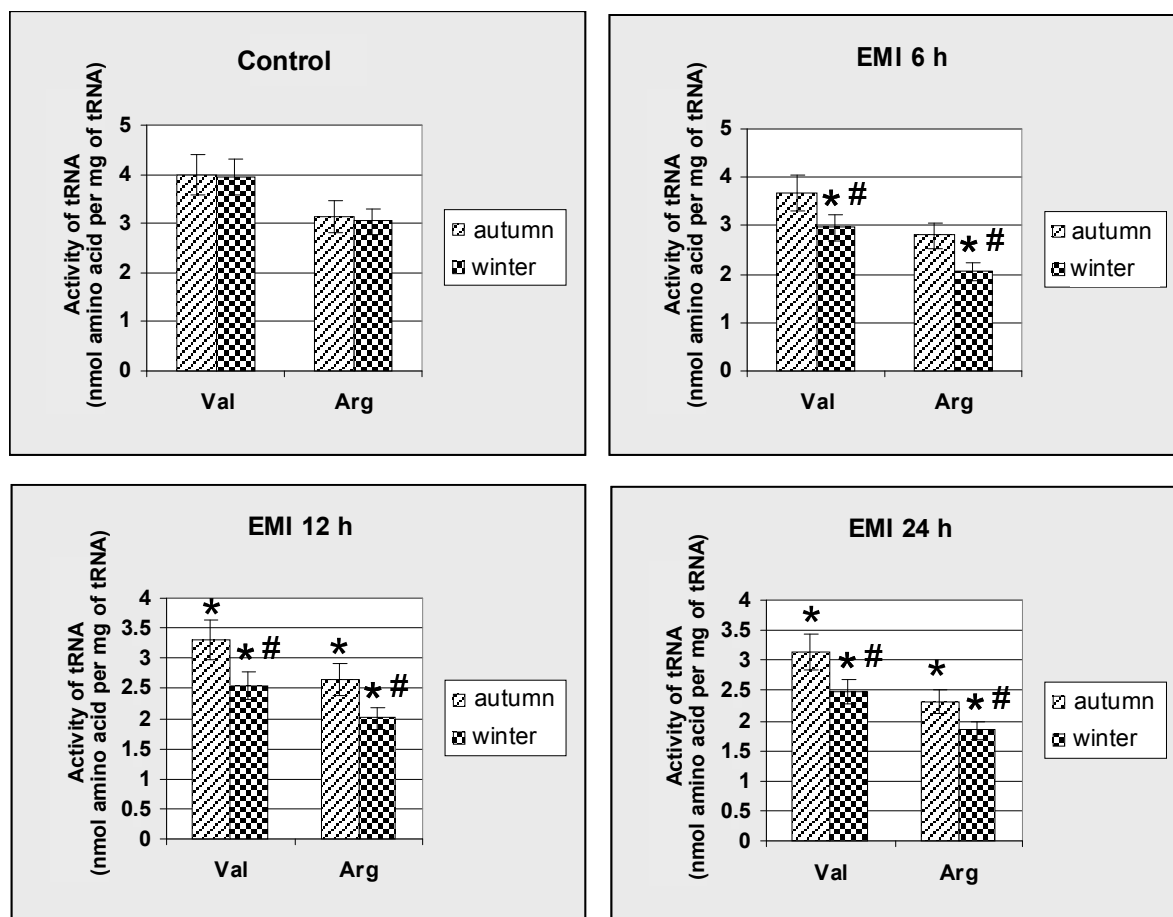


Fig. 1. Acceptor activities of specific tRNA of control rabbit liver and under 6, 12 and 24 h experimental myocardial ischemia (EMI) in autumn and winter

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.

autumn was higher only under 12 h EMI; under 6 and 24 h EMI it remained without changes as compared to control. Activity of arginyl-tRNA synthetase was higher at all durations of EMI as compared to control. Differences of the aminoacyl-tRNA synthetase activities in rabbit liver after EMI in different seasons may be associated with an increase in the activity of inorganic pyrophosphatase which regulates the activity of aminoacyl-tRNA synthetase 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). A lower decrease in the acceptor activity of tRNA and lower increase in the activity of aminoacyl-tRNA synthetase under 6, 12 and 24 h EMI in autumn than in winter may be related to alteration in the actions of some hormones, excretion of which depends on the natural light period of

the day and on differences in the feeding of laboratory animals in different seasons as it has been noted for other subjects (15, 27–29). The decrease of acceptor activity of tRNA under EMI in both seasons studied correlates with the increase in the activity of corresponding aminoacyl-tRNA synthetase which may be part of the compensatory mechanism of the cell to keep synthesis of protein in a normal range under extreme conditions.

Conclusions

1. Acceptor activities of rabbit liver tRNA for valine and arginine under 6, 12 and 24 h experimental myocardial ischemia are different in autumn and winter.
2. Valyl- and arginyl-tRNA synthetase activities of rabbit liver under 6, 12 and 24 h experimental myocardial ischemia are different in autumn and winter.
3. No differences in acceptor activity of rabbit liver tRNA for valine and arginine and in activity of valyl- and arginyl-tRNA-synthetase between control groups

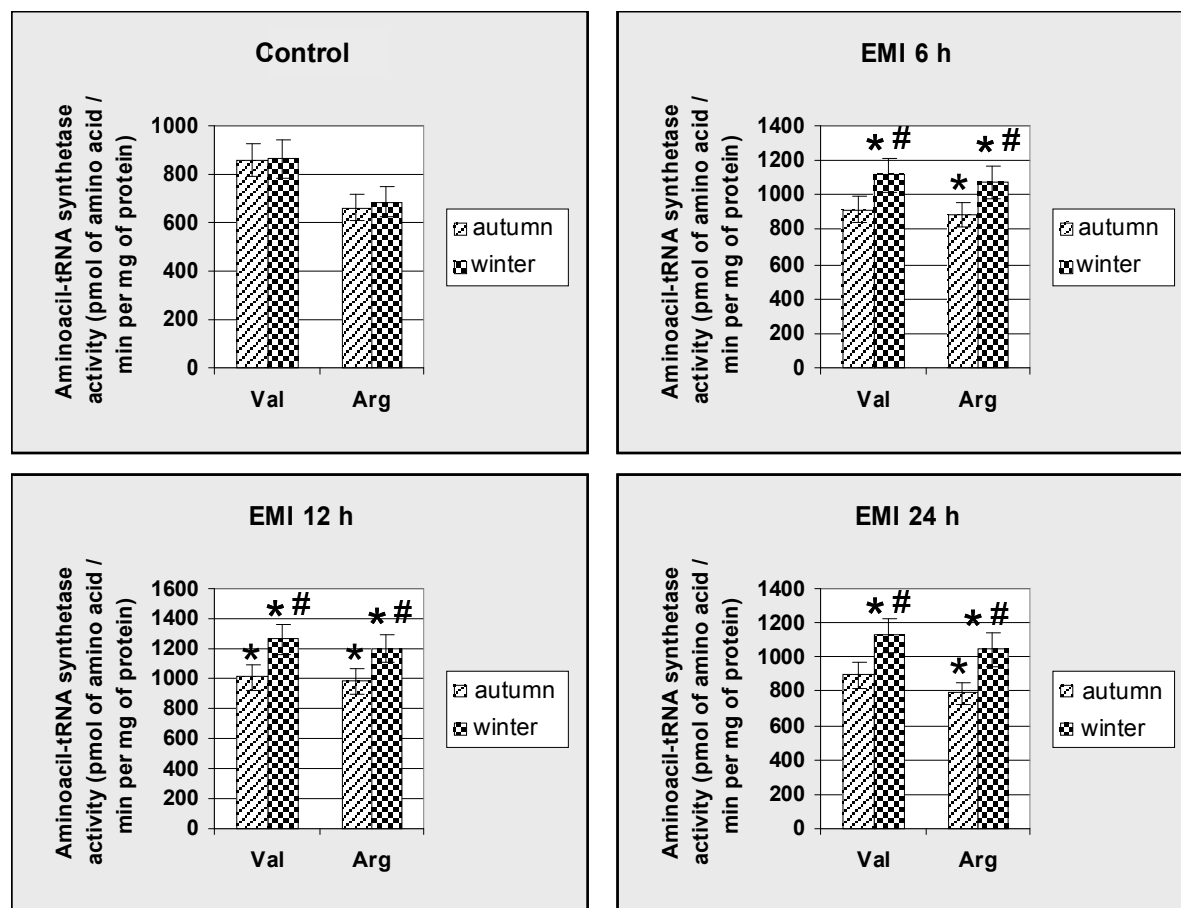


Fig. 2. Activities of specific aminoacyl-tRNA synthetases of control rabbit liver and under 6, 12 and 24 h experimental myocardial ischemia (EMI) in autumn and winter

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.

in autumn and winter were observed.

4. The decrease of acceptor activity of tRNA for valine and arginine under experimental myocardial ischemia correlates with the increase of activity valyl- and arginyl-tRNA synthetases both in autumn and

winter.

5. No differences in acceptor activity of rabbit liver tRNA for valine and arginine and for valyl-tRNA synthetase under 6 h experimental myocardial ischemia as compared to control in autumn were determined.

Triušių kepenų tRNR ir aminoacyl-tRNR-sintetazių specifinių valinui ir argininui aktyvumas, esant miokardo išemijai, skirtingu metų laikotarpiu

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Raktažodžiai: tRNR, aminoacyl-tRNR-sintetazės, baltymų sintezė, metų laikai, miokardo išemija.

Santrauka. Darbo tikslas. Nustatyti sveikų triušių kepenų tRNR gebėjimą prisijungti valiną ir arginą bei praėjus 6, 12 ir 24 val. po eksperimentinės miokardo išemijos sukėlimo ir valil- ir arginil-tRNR-sintetazių aktyvumą skirtingu metų laikotarpiu.

Tyrimo medžiaga ir metodai. Tyrimams naudoti vyriškosios lyties triušiai, sveriantys 2,5–3,5 kg. Ūminė miokardo išemija buvo sukelia perrišus dešinę priekinę nusileidžiančiąją vainikinę arteriją. tRNR ir

aminoacyl-tRNA-sintetazės išskirtos iš sveikų triušių (kontrolinės grupės) kepenų ir praėjus 6, 12 ir 24 val. po eksperimentinės miokardo išemijos sukėlimo rudenį (rugsėjo–spalio mėnesiais) ir žiemą (gruodžio–sausio mėnesiais). tRNA ir aminoacyl-tRNA-sintetazių aktyvumas įvertintas naudojant ^{14}C -aminorūgštis valiną ir arginą.

Rezultatai. Nustatyta, kad rudenį, praėjus 6, 12 ir 24 val. po eksperimentinės miokardo išemijos, triušių kepenų tRNA gebėjimas prisijungti valiną ir arginą yra 24–35 proc. didesnis negu žiemą. Triušių kepenų valil- ir arginil-tRNA-sintetazių aktyvumas, praėjus 6, 12 ir 24 val. po eksperimentinės miokardo išemijos rudenį, yra 15–32 proc. mažesnis negu žiemą. Tiriant kontrolinių grupių triušių kepenų tRNA ir aminoacyl-tRNA-sintetazių aktyvumą skirtingu metų laikotarpiu, skirtumų nerasta.

Išvados. Eksperimentų duomenys rodo, kad triušių kepenų tRNA gebėjimas prisijungti valiną ir arginą bei valil- ir arginil-tRNA-sintetazių aktyvumas, praėjus 6, 12 ir 24 val. po eksperimentinės miokardo išemijos, skirtingu metų laikotarpiu yra skirtingas. tRNA gebėjimo prisijungti valiną ir arginą sumažėjimas po eksperimentinės miokardo išemijos koreliuoja su valil- ir arginil-tRNA-sintetazės aktyvumo padidėjimu tiek rudenį, tiek žiemą. Manoma, kad tai gali būti ląstelės kompensacinio mechanizmo dalis, palaikanti normalaus lygio baltymų sintezę ekstremaliomis sąlygomis.

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References

- Marintchev A, Wagner G. Translation initiation: structures, mechanisms and evolution. *Q Rev Biophys* 2004;37(3-4):197-284.
- Frugier M, Ryckelynck M, Giege R. tRNA-balanced expression of a eukaryal aminoacyl-tRNA synthetase by an mRNA-mediated pathway. *EMBO Rep* 2005;6(9):860-5.
- Park SG, Ewalt KL, Kim S. Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers. *Trends Biochem Sci* 2005;30(10):569-74.
- Schmitt E, Panvert M, Blanquet S, Mechulam Y. Structural basis for tRNA-dependent amidotransferase function. *Structure* 2005;13(10):1421-33.
- Kašauskas A, Viežalienė D, Rodovičius H. Effects of anoxia on pig myocardium tRNA^{Leu} and leucyl-tRNA activities. *Biology (Vilnius)* 2004;2(Suppl 1):60-2.
- Ivanov LI, Martinkus Z, Kharchenko OV, Sara S, Lukoshevichius L, Prashkevichius A, et al. Subcellular distribution and properties of rabbit liver aminoacyl-tRNA synthetases under myocardial ischemia. *Mol Cell Biochem* 1993;125:105-14.
- Rodovičius H. Triušių kepenų tRNA ir aminoacyl-tRNA-sintetazių aktyvumas miokardo išemijos metu skirtingu metų laikotarpiu. (Seasonal differences in activity of tRNA and aminoacyl-tRNA synthetases of rabbit liver in myocardial ischemia.) *Medicina (Kaunas)* 2003;39(1):62-7.
- Kovalenko MI, Rodovitchius GA, Tamulionis AAJ, Pivoriunaite II, Lukoshevichius LJ, Prashkevichius AK. Izuchenije molekuliarnykh osnov narusheniya biosinteza belka pri eksperimental'nom infarkte miokarda i autolize miokarda. (Investigation of molecular fundamentals of protein synthesis disorders under myocardial infarction and myocardial autolysis.) *Molek Biol (Kiev)* 1984;37:18-21.
- Rodovičius H. Miokardo išemijos poveikis triušių kepenų tRNA ir aminoacyl-tRNA-sintetazių aktyvumui skirtingu metų laiku. (Effects of myocardial ischemia on activity of rabbit liver tRNA and aminoacyl-tRNA synthetases in different seasons of the year.) *Biomedicina* 2002;2(2):128-32.
- Rodovičius H, Viežalienė D, Civinskienė G. Activity of tRNA and aminoacyl-tRNA synthetases of rabbit liver under myocardial ischemia in different seasons of the year. *Biology (Vilnius)* 2003;4:7-9.
- Fraser KP, Peck LS, Clarke A. Protein synthesis, RNA concentrations, nitrogen excretion, and metabolism vary seasonally in the Antarctic holothurian *Heterocucumis steineri* (Ludwig 1898). *Physiol Biochem Zool* 2004;77(4):556-69.
- Hew C, Poon R, Xiong F, Gauthier S, Shears M, King M, et al. Liver-specific and seasonal expression of transgenic Atlantic salmon harboring the winter flounder antifreeze protein gene. *Transgenic Res* 1999;8(6):405-14.
- Soveri T, Sukura A, Nieminen M, Lindberg LA. Ultrastructure of the liver of reindeer calves under different nutritional conditions. *Anat Histol Embryol* 1995;24(2):91-5.
- Vera MI, Kausel G, Barrera R, Leal S, Figueroa J, Quezada C. Seasonal adaptation modulates the expression of the protein kinase CK3 beta subunit gene in the carp. *Biochem Biophys Res Commun* 2000;271:735-40.
- Mann DR, Akimbami MA, Gould KG, Ansari AA. Seasonal variations in cytokine expression and cell-mediated immunity in male rhesus monkeys. *Cell Immunol* 2000;200(2):105-15.
- Pickett MH, White BN, Davies PL. Evidence that translational control mechanisms operate to optimize antifreeze protein production in the winter flounder. *J Biol Chem* 1983;258(24):14762-5.
- Toleikis A, Dzeja P, Praskevichius A, Jasaitis AJ. Mitochondrial functions in ischemic myocardium. I. Proton electrochemical gradient, inner membrane permeability, calcium transport and oxidative phosphorylation in isolated mitochondria. *J Mol Cell Cardiol* 1979;11(1):57-76.
- Brungraber EF. A simplified procedure for the preparation of "soluble" RNA from rat liver. *Biochem Biophys Res Commun* 1962;8(1):1-3.
- Choo AHF, Logan DM. Aminoacyl-tRNA synthetases from rat liver: optimized assay conditions and kinetic properties. *Mol Cell Biochem* 1977;17(1):31-8.
- Elska A, Matsuka G, Matias U, Nasarenko I, Semenova N. tRNA and aminoacyl-tRNA synthetases during differentiation and various functional states of the mammary gland. *Biochim Biophys Acta* 1976;247(3):430-40.
- Rodovitchius H, Viezeleiene D, Sadauskiene I, Ivanov L. Protein synthesis and activities of tRNA^{Leu} and leucyl-tRNA synthetase

- in mouse liver under acute lead and zinc exposure. *Trace Elem Electrolytes* 2005;22(4):288-91.
22. Rodovičius H, Vieželiene D, Sadauskienė I, Valentukonytė S, Ivanov L. The effects of zinc ions on activities of tRNA^{Leu} and leucyl-tRNA synthetase of mice liver. *Medicina (Kaunas)* 2004;40(10):982-6.
23. Lukoshevichius LJ, Rodovitchius GA, Kovalenko MI, Pivoriunaite II, Prashkevichius AK, Elskaja AV. tRNK i aminoacil-tRNK sintestazy pecheni krolikov pri eksperimental'nom infarkte miokarda. (tRNA and aminoacyl-tRNA synthetases from liver tissue of rabbits under experimental myocardial infarction.) *Vopr Med Khimii* 1983;29(4):65-9.
24. Rodovitchius GA, Kovalenko MI, Ivanov LJ, Lukoshevichius LJ, Elskaja AV. Aminoacil-tRNK sintestazy pecheni krolikov pri eksperimental'noj ishemii miokarda. (Rabbit liver aminoacyl-tRNA synthetases under experimental ischemia of myocardium.) *Dokl AN USSR Ser B* 1982;4:65-8.
25. Vieželiene D, Ivanov LL, Rodovičius H, Praškevičius A. The activity and aggregate state of rabbit liver aminoacyl-tRNA synthetases and tRNA methyltransferases under myocardial ischemia. *Biology (Vilnius)* 1995;1-2:83-5.
26. Nathanson L, Deutscher MP. Active aminoacyl-tRNA synthetases are present in nuclei as a high molecular weight multienzyme complex. *J Biol Chem* 2000;275(41):31559-62.
27. Sweeney T, Kelly G, O'Callaghan D. Seasonal variation in long-day stimulation of prolactin secretion in ewes. *Biol Reprod* 1999;60(1):128-33.
28. Sohn YC, Yoshura Y, Kobayashi M, Aida K. Seasonal changes in mRNA levels of gonadotropin and thyrotropin subunits in the goldfish, *Carassius auratus*. *Gen Comp Endocrinol* 1999;113(3):436-44.
29. Sen U, Mukherjee D, Bhattacharyya SP, Mukherjee D. Seasonal changes in plasma steroid levels in Indian major carp *Labeo rohita*: influence of homologous pituitary extract on steroid production and development of oocyte maturational competence. *Gen Comp Endocrinol* 2002;128(2):123-34.

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