ISSN 0012-4966, Doklady Biological Sciences, 2016, Vol. 466, pp. 42–44. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © L.V. Chernaya, L.A. Kovalchuk, E.S. Nokhrina, 2016, published in Doklady Akademii Nauk, 2016, Vol. 466, No. 6, pp. 744–746.

GENERAL BIOLOGY

Role of the Tissue Free Amino Acids in Adaptation of Medicinal Leeches *Hirudo medicinalis* L., 1758 to Extreme Climatic Conditions

L. V. Chernaya, L. A. Kovalchuk, and E. S. Nokhrina

Presented by Academician V.N. Bol'shakov July 27, 2015

Received September 28, 2015

Abstract—The first comparison of the spectra of free amino acids in tissues of the medicinal leeches *H. medicinalis* from different climatic and geographical Eurasian areas has been performed. Adaptation of *H. medicinalis* to extreme climatic conditions occurs via intensification of the amino acid metabolism resulting from a significant increase in the content of essential amino acids. Accumulation of arginine, histidine, and lysine (3.6-, 3.9-, and 2.0-fold increases, respectively) has proved to play a special protective role in adaptation of *H. medicinalis* to the low positive temperatures.

DOI: 10.1134/S0012496616010129

Medicinal leeches *Hirudo medicinalis* L., 1758, which are the Palaearctic endemics, inhabit mainly the European water bodies. The latitudinal spread of this species range (from the British Isles in the west to the Altai Mountains in the east) covers at least 5700 km and is characterized by a great diversity of the microclimatic conditions, depending on the land form and the water-body type, as well as on the climate continentality [1, 7]. Changes in climatic conditions in the direction of decreasing average annual temperature are the main limiting factor for the distribution and abundance of *H. medicinalis*. These leeches have a southern origin, and the ground freezing is rather dangerous for them; the temperature dependence plays a decisive role for their reproduction and survival [1]. Many water bodies are believed to be an unsuitable habitat for *H. medicinalis* because of low water temperature [4]. When the biologically active compounds protecting cell membranes from the low-temperature functional destabilization are present in tissues, they are known to promote a biochemical adaptation of the poikilothermic animals to the low-temperature habitats [2, 4–6].

The goal of this study was to determine the role of free amino acids in tissues of the medicinal leeches during their adaptation to the extreme conditions of various climatic and geographical areas.

Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia
e-mail: kovalchuk@ipae.uran.ru; chernaya_lv@mail.ru

We have carried out the first study on the content of amino acids in tissues of adult leeches *H. medicinalis* caught during their exit from winter hibernation in six water bodies of the following climatic and geographical areas: the regions of the European part of Eurasia (Tambov oblast, the Lesnoi Voronezh River; Lugansk oblast, Lake Glubokoye; Kharkov oblast, Lake Goreloye and the Udy River), where the average annual temperatures range from +6.1 to +11.9°C; Western Siberia (Altai krai, Lake Damba and the Togul River) with the average annual temperature of +2.6°C. The concentration of free amino acids (AAs, μmol/100 g tissue) in the skin and muscle tissues of leeches was determined by the ion-exchange chromatography on a AAA-339M automatic amino acid analyzer (Microtechna, Czech Republic) [3]. Processing of the experimental data has been performed using the Statistica 6.0 applied package. The differences were regarded as significant at $p \le 0.05$ according to Student's t test.

The amino acid spectrum in tissues of *H. medicinalis* is represented by 21 AAs (table). Glutamine, glutamic acid, alanine, asparaginic acid, and glycine were the dominating amino acids irrespective of the climatic belt inhabited by the medicinal leeches; the content of these AAs ranged from 30.5 to 35.9, 13.8 to 12, 22.4 to 26.4, and 6.2 to 4.3%, respectively. In no case have we found significant influence of the climatic and geographical conditions of the aquatic ecosystems inhabited by *H. medicinalis* on the tissue content of glutamic acide and glutamine playing the key role in nitrogen metabolism, metabolically active alanine, which is involved in glucose synthesis, or the sulfur-containing cysteine and methionine, AAs with antioxidant properties ($p > 0.05$). Taurine and tryp-

The content of free amino acids in tissues of *H. medicinalis* from different climatic and geographical areas

Amino acid	European *H. medicinalis* ($n = 40$)	Siberian *H. medicinalis* ($n = 20$)	p
Cysteic acid	19.1 (16.0–22.1)	42.9 (34.2–51.8)	<0.001
Asparaginic acid	391.2 (375.7–406.5)	559.4 (539.9–578.9)	<0.001
Threonin	45.4 (39.6–51.2)	66.3 (63.1–69.5)	<0.001
Serine	66.8 (59.7–73.9)	109.6 (104.6–114.5)	<0.001
Glutamic acid + glutamine	626.5 (588.9–664.0)	646.7 (637.0–656.3)	0.450
Proline	29.1 (24.1–34.1)	1.34 (1.22–1.47)	<0.001
Glycine	108.9 (104.6–113.1)	92.1 (88.1–96.1)	<0.001
Alanine	240.3 (226.7–253.9)	253.6 (246.5–260.7)	0.184
Valine	44.0 (38.1–49.9)	73.7 (71.4–76.1)	<0.001
Cysteine	5.99 (4.67–7.32)	4.32 (4.12–4.52)	0.077
Methionine	13.5 (12.4–14.6)	15.1 (13.7–16.5)	0.083
Isoleucine	16.7 (15.3–18.1)	37.0 (35.2–38.9)	<0.001
Leucine	53.8 (51.1–56.4)	79.3 (74.8–83.7)	<0.001
Tyrosine	10.6 (9.56–11.7)	16.9 (16.3–17.7)	<0.001
Phenylalanine	21.7 (19.8–23.7)	38.2 (35.5–40.9)	<0.001
γ-aminobutyric acid	5.09 (4.45–5.73)	3.05 (2.05–4.04)	<0.01
Ornithine	17.8 (16.9–18.7)	16.3 (15.9–16.6)	<0.05
Lysine	24.8 (22.1–27.4)	50.3 (46.3–54.2)	<0.001
Histidine	2.04 (1.82–2.25)	7.94 (7.47–8.42)	<0.001
Arginine	1.49 (1.01–1.99)	5.29 (4.42–6.16)	<0.001
AA pool	1744.8 (1663.4–1819.0)	2119.4 (2064.6–2174.3)	<0.001
Essential AA	223.4 (205.2–241.6)	373.1 (357.8–388.5)	<0.001
Nonessential AA	1479.4 (1408.7–1550.1)	1683.9 (1650.8–1717.2)	<0.001

The average values of amino-acid concentrations are presented (μmol/100 g) as well as 95% confidence intervals (in parentheses); n, the number of leeches per group.

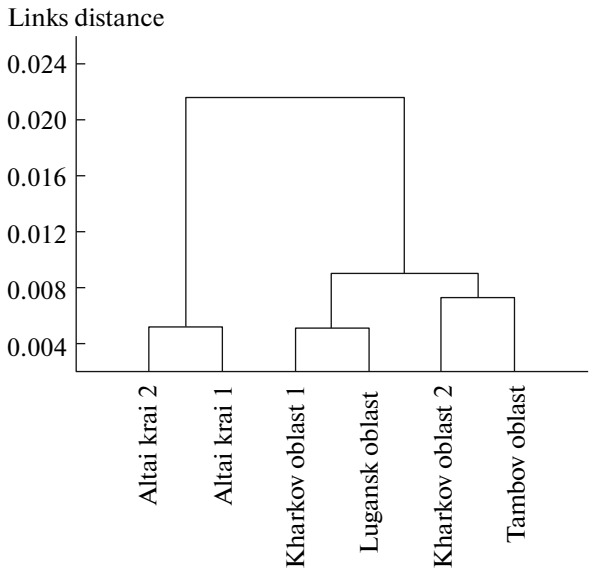
tophan have not been identified in the leech tissues (table).

Significant differences of quantitative distribution of free essential and nonessential AAs in tissues of the medicinal leeches from different climatic and geographical areas were determined by the method of cluster analysis (figure). The leeches inhabiting Western Siberia differ from those of the aquatic ecosystems in the European part of Eurasia by the high level of the overall pool of free amino acids (table, $p < 0.001$). At low positive temperatures, the tissues of Siberian leaches contain much higher concentrations of essential AA (lysine, threonine, valine, isoleucine, leucine, phenylalanine, histidine, and arginine; $p < 0.001$) than the tissues of European leeches. High concentrations of arginine, histidine, and lysine (a 3.6-, 3.9-, and 2.0-fold increase, respectively) proved to play a special protective role in adaptation of the medicinal leeches to low positive temperatures under the environmental conditions of Western Siberia.

When compared with the leeches from the European water bodies, the tissues of Siberian leeches contained reduced concentrations of glycine, γ-aminobutyric acid, and ornithine (by 15, 40, and 8%, respectively) (table, $p < 0.05$). A significant (21.7-fold) decrease in the concentration of proline in *H. medicinalis* from Siberian water-bodies as compared to the European specimens is, probably, an element of their compensatory response to the conditions of their habitat.

Our experiments suggest that intensification of the AA metabolism through a considerable increase in the essential AA pattern is one of the adaptation mechanisms of *H. medicinalis* to the climatic conditions of Western Siberia (severe winters, a short frost-free period, deep soil freezing at the annual average temperature of +2.6°C at the western border of the area).

An increase of the overall pool of free amino acids in Siberian leeches supports their survival at high amplitude of fluctuations of the near-zero temperatures of the environment.



Dendrogram of the amino-acid spectrum similarity in tissues of *H. medicinalis* from different climatic and geographical areas.

It has been shown that an increase in the tissue concentrations of essential AAs (lysine, arginine, and histidine) is a specific feature of the medicinal leeches from Siberian water bodies. These AAs are involved not only in energy metabolism, but also in the mechanisms of adaptation to low positive temperature, and they play a cryoprotective role to protect cell structures from hypothermal destabilization.

The results of this study suggest that an increase in the content of some essential AAs is a specific factor that promotes the low-temperature adaptation and, hence, survival of leeches under the conditions of the cold Siberian climate.

REFERENCES

1. Lukin, E.I., *Piyavki presnykh i solonovatykh vodoemov* (Leeches of Fresh and Brackish Waters), Leningrad, 1976, vol. 1.
2. Karanova, M.V., *Izv. Akad. Nauk, Ser. Biol.*, 2006, no. 6, pp. 719–724.
3. Koval'chuk, L.A., Chernaya, L.V., and Nokhrina, E.S., *Vopr. Biol. Med. Farm. Khimii*, 2011, no. 6, pp. 61–64.
4. Elliott, J.M. and Tullett, P.A., *Biol. Conservation*, 1984, vol. 29, pp. 15–26.
5. Fields, P.G., Fleurat-Lessard, F., Lavenseau, L., et al., *J. Insect Physiol.*, 1998, vol. 44, pp. 955–965.
6. Karanova, M.V., *Biol. Bull.*, 2011, vol. 38, no. 2, pp. 116–124.
7. Utevsky, S., Zagmajster, M., Atemasov, A., Zinenko, O., Utevska, O., Utevsky, A., and Trontel, P., *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 2010, vol. 20, pp. 198–210.

Translated by A. Nikolaeva